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A STUDY OF VARIATION IN THE SIAMESE FIGHTING FISH, *BETTA SPLENDENS*, WITH EMPHASIS ON COLOR MUTANTS AND THE PROBLEM OF SEX DETERMINATION

by

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INTRODUCTION

Species of small fishes suitable for rearing in aquaria have long been a popular diversion. They are also used as experimental animals in a variety of ways, and are kept in many biological laboratories because of their educational value as demonstration material.

While the art and science of pisciculture is known to have been practiced by the Romans and early Orientals, aquarium keeping as we know it today traces back to about 1850. Commercial interests estimate that the hobby has grown most rapidly in the last 20 or 30 years. As many as 20,000,000 Americans now participate. Aquarium keeping has gained recognition as one of the top ranking hobbies in terms of numbers of people involved and amounts of money expended annually.

Research uses of these fish also have expanded greatly. For example, aquarium fishes serve as useful substitutes for larger, more expensive, and more difficult to keep game species in almost every respect. Methods of stimulating reproduction, handling of eggs and fry, nutritional and growth problems, transportation, control of disease and parasites, and population dynamics are some which may be economically studied with species better adapted to aquarium life. Principles worked out for these may be perfectly suitable for larger types.

Naturally, some aspects of fish research are not adaptable to this kind of study. Useful information is obtained from both areas.

One example is the widespread use of special packaging and shipping techniques which are essentially the product of research on the transportation of game fish to stocking areas. The major fishery research areas have also yielded most of the primary results in nutrition and endocrinology. It is obvious that both areas of study can complement one another.

Of the aquarium fish used for research a few have received concentrated attention and have yielded considerably more information than the others. Among the best known are several members of the genus Poecilia including the Mollies and the common Guppy, Poecilia reticulata. The Platyfish, Xiphophorus maculatus and the Swordtail, Xiphophorus helleri are others of the ovoviviparous (live-bearing) forms popular with aquarists. Better known egg-laying forms include the Zebra fish, Brachydanio rerio, the Japanese Rice fish (Medaka) Oryzias latipes, the long-popular Goldfish, Carassius auratus, and the Siamese Fighting Fish, Betta splendens.

Studies of fish genetics, using aquarium species, have provided interesting information which has contributed greatly to general genetical knowledge. Of particular interest has been the work of Dr. Myron Gordon over many years. It has dealt with polymorphic natural populations of Xiphophoran fishes, their various systems of sex determination, interspecific hybridization within the genus, and the effects of various genetic backgrounds on the development of various pigment cells. The pigment cell research led to important studies dealing with normal and abnormal pigment cell physiology and genetics of cancer. The work of Winge (1934) concerning the experimental alteration of the sex

determining system in guppies from male to female digamety and studies involving mapping of sex-linked color-determining loci and crossing over between the sex chromosomes have also been significant.

Betta splendens Regan

The so-called Siamese "fighting fish", Betta splendens Regan, is one which has attracted more than the average amount of interest because of its convenient size, its general hardiness, its very noticeable physical, physiological and behavioral characteristics, and especially its spectacular color variation. Literally million of Bettas are produced each year in the United States for aquarists who have made them one of the top ranking species in terms of popularity.

Bettas have been of scientific interest for a number of years. Dr. Hugh M. Smith (1945) produced an exhaustive monograph on the fresh water fishes of Siam (Thailand). He served as U.S. Commissioner of Fisheries from 1913 to 1922 and as Advisor in Fisheries to the Siamese Government from 1923 to 1935. From 1922 until his death in 1941 he was also Associate Curator in Zoology in the U.S. National Science Museum. In his monograph he says of the Betta (p. 456):

> For several hundred years the fish has been used locally (Siam) for sporting purposes, and for 90 years it has been domesticated and cultivated.

In this same account Smith referred to earlier writings of his own (1937a, 1937b) from which he abstracted the following:

> Just how early in Siamese history the fighting fish acquired its reputation is not known, but for several hundred years its

pugnacious qualities have been recognized and utilized in popular contests.

Up to the year 1850 or thereabouts, the use of the fighting fish in sportive contests in Siam was confined to fishes obtained in open waters, but in order to insure a regular supply for fighting and betting purposes, domestication and cultivation were then instituted and have since been conducted on an increasing large scale. It may be noted, however, that in recent years cultivation has been less important as a factor in fighting contests and has represented a better appreciation of the fish's beauty of color and form.

It appears that Bettas were only kept for fighters originally and breeding efforts were undoubtedly inclined toward improvement of fighting quality through selection for increased aggression and stamina. Later, new variations in color and form turned up and these aspects became the objects of genetic investigations (Goodrich, 1934; Umrath, 1939; Eberhardt, 1941; Wallbrunn, 1948, 1958), embryology (Van Duijn, 1938), and sex determination (Eberhardt, 1943b; Wallbrunn, 1951). Recently, attention has been directed to studies of Betta behavior (Braddock, _et al._, 1955, 1959, 1960, 1961; Hess, 1952; Ketusink and Chepanich, 1963; Marrone, 1965), schizophrenia (Smith and Moody, 1956) the effects of various pharmacological agents (Abramson, 1954, 1957; Keller, 1959; Muller, 1959; Smith 1956; Trout, 1957; Turner, 1956; Weiss, 1958), and other physiological aspects (Adler and Hogan, 1963; Goldstein, 1967; Thompson, _et al._, 1963, 1965a, 1965b, 1966).

As my own investigations progressed I found that the genetic information concerning this species had been cursory and needed substantial revision. Abnormalities in domestic stocks include several which lack any genetic analysis, and some which have been studied but never reported in scientific literature.

None of the previous workers have attempted to interpret phenotypes in terms of mutational origin. A primary difficulty is the fact that descriptions of wild-type Bettas have been confused. Smith (1945, p. 456) has the following remarks, which are, indeed, colorful:

> The general coloration of a quiescent fish is a dull grayish brown or green with or without obscure dark lateral bands, and conveys no suggestion of the wonderfully brilliant hues assumed by the male under proper stimulation. Under the stress of excitement the male fish exhibits a remarkable change. All the fins are widely spread, the gill membranes are expanded and project like a frill or ruff suggestive of the raised hackles of fighting cocks, and the entire body and fins become intensely suffused with a lustrous blue or red color, which makes the fighting fish one of the most beautiful of all fresh water fishes..... A person seeing for the first time a wild fighting fish would never suspect the wonderful possibilities in coloration that have been realized under cultivation.

Because of Smith's unquestioned authority his descriptions have been accepted generally without question and are usually reiterated, at times with embellishment, even today. An example taken from a rather substantial popular book is that of Sterba (1966, p. 785):

> Very variable in colouring, in relation to the wide distribution. Fine specimens are red-brown with strong blue-green glint and numerous metallic spots, usually arranged in rows and green in colour but occasionally also blue or red. Dorsal fin red-brown, with a brilliant green stripe and a chessboard pattern on the hinder part. Caudal fin red-brown, with a fan-like pattern of green stripes and usually with an orange edge. Anal fin blue-green with brown and reddish stripes. Ventral fins fiery red with white tips. Young males similar to females. Females yellowish brown with faintly indicated transverse bands and often with longitudinal bands closely approaching to a golden colour. All fins yellowish green with a narrow red border.

The descriptions suggest, in the case of "alternating.....stripes" for example, that there may be areas where one type of color is present

and other areas which have another. Actually, "green stripes" are the effect of an opaque, reflective layer of iridocyte color which is concentrated along the rays of the fins. The alternative color is not similarly localized but only appears to be. Normal fins are uniformly covered with red pigment cells and would appear not striped but a solid red color if the opaque contrasting stripes did not hide part of it. Sterba's "Anal fin blue-green with brown and reddish stripes" gives no indication that he had more than a superficial understanding of the colorations. In the absence of rational descriptions, it is not surprising that attempts to analyse the color genetics of Bettas in the past have been spasmodic and confused.

During the course of these investigations I have established a more reliable description of a standard color based on the kind, relative abundance, and distribution of the different pigment cells, and have attempted to work out a sensible method of categorizing phenotypes based upon combinations of the various color producing elements. I have made a series of genetic tests involving the color elements mentioned. These included previously-described types as well as new varieties which are now available. In addition, the symbolism for genetic factors in the Betta has been reviewed and revised in recognition of modern practices.

An additional problem of genetical significance impressed me during early stages of this study. This was the extraordinary sex ratios obtained, suggesting peculiar factors in sex determination. While this problem had been recognized by some previous investigators no extensive studies of it had been made. Early matings were made in

the Genetics Laboratory at Iowa State University. Later, the project was moved to my residence near Ankeny, Iowa. After a few spawns had been reared at Ankeny a very striking shift was noted. In Ames, all spawns but one (which could be discounted since it contained only three individuals) contained an excess of females. In Ankeny the situation was sharply reversed. The Ames spawns contained about twice as many females as males whereas the Ankeny spawns produced nearly six times as many males as females!

In hopes of screening out a possible influencing factor related to sex determination an experiment was designed to analyze some of the variables which were suspected of having significance.

This report, therefore, is an attempt to modernize the genetics of the Betta. Some of the problems and findings may have valuable general significance in Biology. The fish has great potential for exploitation in the commercial-hobby field, teaching-demonstration, and experimental areas, especially of fish genetics and developmental anatomy and physiology. A more solid genetical base will help pave the way to further success in these areas.

LITERATURE REVIEW

The Siamese Fighting Fish, Betta splendens Regan, is one of a number of "air breathing" fishes of the order Labyrinthici. Labyrinth fishes are characterized by the possession of a respiratory modification in the form of an air chamber filled with plates or leaflets of respiratory epithelium located above the gills and functioning as adjuncts to them in obtaining oxygen. This accommodation and variations of it are not uncommon in organisms which have become adapted to water which is frequently low in oxygen content.

Taxonomy and Origin

Until recently the family classification for the Betta was Anabantidae. Anabantid fishes are found throughout southeastern Asia as far north as northern China and Korea and including the Philippines and the Malayan archipelago to, and through, southern and western Africa. The Asian and African forms are somewhat distinct and the African forms are not as well known. Members of the genus Betta are Asian. Some are widely distributed but, according to Smith (1945), Betta splendens appears to be found in the wild state only in Siam (Thailand). It may be that they were widely distributed artificially for possible mosquito control at an early date, making it difficult to accurately determine their natural range.

The best account of the fishes of Siam (Thailand) is that of Smith (1945). Smith's review summarizes the taxonomical data concerning the

Betta. He indicated that the first reference to freshwater Siamese fishes was an ichthyological report in 1831 by Cuvier and Valenciennes. Pieter Bleeker wrote of Siamese fresh water fishes as early as 1850.

The earliest reference to what I believe to be Betta splendens is attributed to Cantor (1850) who used the name Betta pugnax. The generic name is attributed to Bleeker. The type specimen of the genus is Betta trifasciata Bleeker (Smith, 1945, p. 454). This specific name is now replaced by Betta taeniata Regan. Jordan (1963, p. 245) has only the entry "Pieter Bleeker. Betta Bleeker, XXIII, 12, Orthotype B. trifasciata Blkr." Regan (1910) noted that the Siamese form was not Betta pugnax but a distinct species. More recently, Liem (1963) reclassified anabantid fishes on an osteological basis and sub-grouped Betta splendens with the Belontiidae which he considered a separate family from the Anabantidae. Bettas remain well-known, however, as "Anabantid fishes".

Smith (1945) stated that there are a dozen or more species of Betta inhabiting Borneo, Sumatra, Java, and Malaya but only two closely-related forms, Betta splendens and Betta taeniata, are found as far north as Thailand. Kuronuma (1961), in a check list of the fishes of Vietnam, lists a "Fighting Fish" identified as Betta pugnax Cantor.

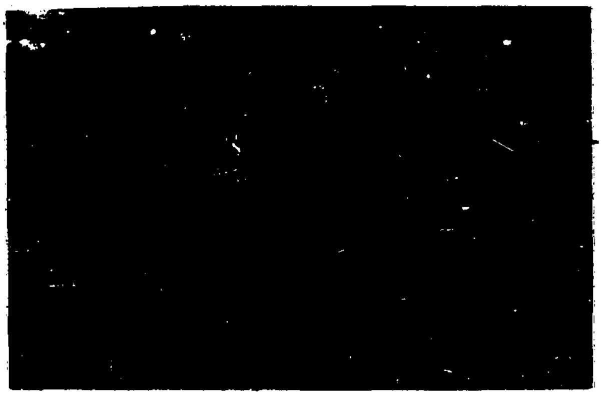
In early 1968 I obtained some wild Bettas from Vietnam. Dr. George S. Myers, who has considered Betta taxonomy in the past (1922, 1929) in a personal correspondence discussed the proper identification of these fish. He indicated that Betta pugnax is found only on the island of Pinang, as was suggested by Smith, and suggests that the form being referred to as Betta pugnax may be synonomous with his (Myers') Betta

taeniata.

The Vietnamese fish which I obtained are shown in Figure 1. They not only look as they were expected to but they also spawn in the same manner and cross readily with domestic fish. I am confident, therefore, that they are not *Betta taeniata*. The natural range of *Betta splendens* has never been accurately determined but geographical features are such that it would be perfectly reasonable to find them in Vietnam.

Part of the reason for lack of accurate historical information is that early domestic varieties were first identified and casually named as new species rather than as color variation. Such descriptive names as *Betta cambodia* (or *cambodja*), *Betta cyana*, *Betta rubra*, and *Betta splendens*, var. *longicauda* certainly did not help communication in the early days of Betta breeding. The fact that these names were used indicates that these varieties were already extant at a relatively early time. The modern understanding that they are ordinary mutants leads one to believe that the fish had already been under cultivation for sufficient time and with enough attention that "sports" had been found and preserved by the breeders.

Of the various genera in the Belontiidae there are two which are superficially very much alike. These are *Trichopsis* and *Betta*. In both genera the dorsal fin originates far behind the base of the pectorals and the dorsal is normally shorter than the anal fin. The pelvic, or ventral, fins have their first ray extended into a filament. The genus *Betta* has a well developed spine in the ventral fin, a vestigial lateral line, an entire preorbital (as opposed to a serrate one in *Trichopsis*), 2 to 4 dorsal spines and 1 to 4 anal spines. Of the two Thai Betta



a

a. Wild type male in quiescent state. Domestic male in background. Note color and short fins.

b

c

b. (left) Wild type male in aggressive display. Note darkening color of body

c. (right) Wild type male in aggressive display showing darker color on areas of the body. The body may become almost black.

Figure 1 Wild <u>Betta splendens</u>

species described by Smith (1945) the following data are given:

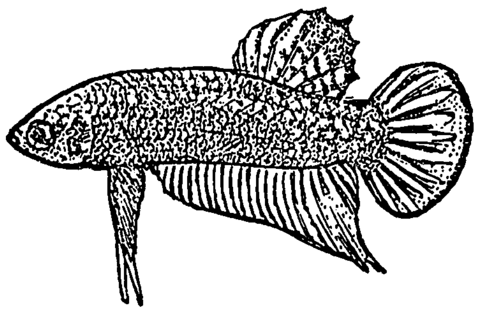
> taeniata: Head broad; interorbital space wide and flat; anal rays II, 20 to 25; caudal rays produced; back bluish black, sides light brown, belly whitish; 2 or 3 blackish longitudinal bands, one from snout to base of caudal, one from under eye across opercle and thence along base of anal fin, one (often indistinct in life) from upper edge of eye to upper part of caudal peduncle; gill membranes light colored; dorsal fin light brown, with obscure lines of dark spots; caudal and anal fins brown; ventral and pectoral fins whitish.
>
> splendens: Head less broad; interorbital space narrow and convex; anal rays II to IV, 21 to 24; caudal rays not produced; darkish green above, red below, scales dark edged; a dark, oblique stripe from eye to subopercle, sometimes 2 dark longitudinal bands from eye to base of caudal; gill membranes blackish; rays of dorsal fin dark, membranes green with dark undulating stripes; caudal rays red, membranes green; anal and ventrals red; pectorals pale.

It would be well to point out at this time that this information may be satisfactory in distinguishing between these two species for taxonomical purposes but the color descriptions are only superficially correct, as has been mentioned previously. General anatomical comparisons of wild and domestic types of Betta splendens are shown in Figure 2.

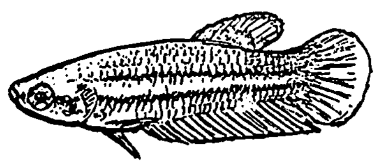
Domestic variation

According to Sterba (1966), they were bred by Juenot in 1892 or 1893. They were first brought into the United States in 1927 by Mr. Frank Locke, a dealer-importer in San Francisco. Others were brought into the country by Dr. Smith at about the same time.

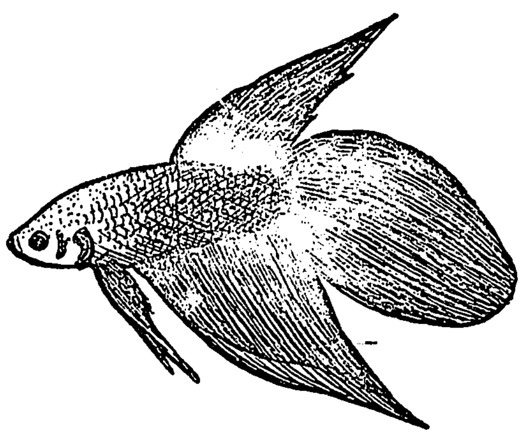
It appears that Bettas were first propagated in captivity for purposes of producing stocks of fighters. Breeders noted other things in the fish than fighting ability. Smith (1945, p. 457) offers the



a. Wild type male Betta splendens



b. Wild type female Betta splendens



c. Domesticated, long finned male Betta splendens

Figure 2 Morphological variations in Betta splendens

following concerning breeding of the species:

> Even in the hands of persons ignorant of the laws of heredity, noteworthy improvements in form, size, coloration, and fighting ability have been brought about; and there is reason to believe that still further improvements may be made.....in addition to intensified reds and blues, are lavenders, iridescent green, cornflower-blue, blue and white, and yellowish and reddish creams with bright red fins. The latter, first produced around 1900, are known to the Siamese as Pla Kat Khmer (Cambodian Biting Fish), probably from having originated among fanciers in French Indo China.....along with the development of intensified and new colors, there has come about an increase in the size of the vertical fins culminating in crapelike effects, which vie with those in the veil-tailed and other highly cultivated Japanese goldfish, so that there are now fighting fish whose caudal fins are about as long as the head and body combined.

Sterba's description of domesticated forms in a well regarded hobbyist guide, is similarly effusive (1966, p. 786):

> Numerous long finned varieties of Betta splendens have been developed under domestication, using the techniques of selective breeding and isolation to produce forms in which enhanced beauty of colouring is allied to extremely long finnage. These are now available in a great variety of colours: white with iridescent blue flanks, emerald green, cornflower-blue, wine-red, brick-red, black, etc.

There can be no question of the interesting variety available for study in color and morphology of this species.

Color and Pigmentation

It is necessary to recognize that there are certain elements which basically contribute color and that there are certain physiological states of the fish which may substantially alter the basic appearance. Generally, fish in good condition have their colors at good intensity and those in

poor condition are pale. Within the range of their general condition they can "fade" or "intensify" in response to certain neurological or hormonal stimuli.

Variations caused by normal shifts in melanophores in Bettas are shown in Figure 3. These might be divided into a light, or quiescent, phase and a dark, or excited, phase. Certain patterns are normally associated with these phases. Usually, both sexes show longitudinal striping, the stripes being formed by alternating regions of dense and sparse dark pigment cells. When the fish are in certain physiological states they may darken somewhat, through expansion of pigment in other cells, until they appear uniformly colored. In display condition, males become very dark, almost black and appear to lose any sign of a striped or banded pattern. Females in this condition usually show a series of alternating transverse light and dark bands which have been associated with a submissive condition as when they are ready to spawn. On rare occasions the transverse banding is shown by males, especially if they have been bested in a fight. The color and pattern changes have been of interest to behavioral students, as has been mentioned previously There are some color mutants included in Figure 3 but these are of no concern at this point.

Animal coloration

The literature dealing with animal coloration is vast and much is known of the basic elements, at least, of pigmentation. Parker (1948), Fingerman (1963), and Waring (1963) have produced monographic works

Figure 3. Variations in dark coloration of domestic _Betta splendens_

a. Young male showing wild type coloration

b. Older male showing wild type coloration, longer finnage common to domesticated stocks, and display attitude

c. Mature female showing wild type coloration and longitudinal striping

d. Mature female showing a different color phenotype with normal longitudinal striping

e. Mature female from a dark strain showing transverse banding exhibited normally by females during spawning behavior or pre-spawing display

f. Same female in a more quiescent state and showing color change to longitudinal striping

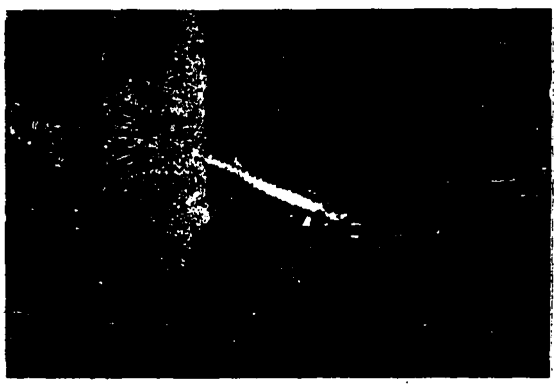
g. Mature female of blue phenotype but normal for dark pigment and showing transverse banding

h. A mature pair, the female (left) is from a dark race and in her present condition is so dark as to nearly obliterate banding patterns, the male (right) exhibits transverse banding to an unusual degree for his sex

a



b

c
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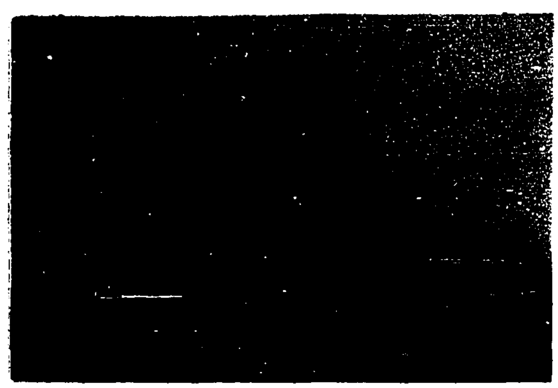
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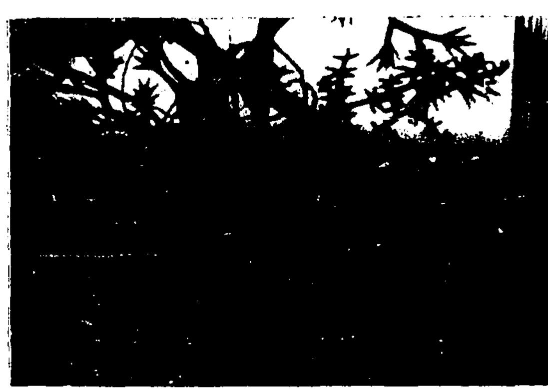
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dealing with physiological aspects of color, particularly in the area of controls. They all provide information concerning color control in fishes but their presentations generally concern adaptation and protective coloration with little indication of genetic aspects. Both Fingerman and Waring state that pigment granules in melanophores may be concentrated or dispersed producing general lightening or darkening. A variety of methods of control are used, neural, hormonal, or combinations of the two.

The following discussion of elements thought to be involved in some way with Betta pigmentation has been selected from a review by Fox (1953).

Animal colors were considered to be of two basic types: 1) true pigments, of chemical nature, involving the absorption of one or more of the components of white light, and called "Biochromes", and 2) "Schemochromes", which are physical (structural), where some object in some way diffracts light rays. Generally, the schemochromes do not exist without the presence of a biochrome which enhances or modifies in some other way the visible effect of the schemochrome.

There is a definite relationship between metabolic activity and the biochromes. This is probably also true of some schemochromes, at least in an indirect way. The biochromes may in many cases be end products of various general metabolic processes indicating that color and biochemical activity are often intimately related.

It should be recalled that there are certain basic physical factors involved in our perception of color. Essentially, we see opaque colors as incident or reflected light and transparent colors by transmitted, or

combinations of transmitted and reflected, light. Objects are said to be black if they absorb all wave lengths of light and reflect none. They are white if they reflect all wave lengths. Greys are produced when all wave lengths are partially absorbed in approximately equal amounts. We see color only when there is selective absorption and various penetration. Green looks green, for instance, when the object we call green absorbs all wave lengths except green and green is reflected and observed.

The various physical effects which may influence light waves can be summarized briefly as simple reflection, which creates whites such as is seen in snow; refraction, which involves passage of the rays through some prismatic structure which bends the rays and which yields the various colors in rainbows, ordered as they are because of different relative wave lengths; diffraction or scattering, with degrees of polarization, which creates discontinuities within otherwise transparent media; interference, the iridescent changeable colors resulting from non-synchronous reflection of waves from various layers in transparent, laminated substances; and absorption, where some or all color elements may be completely absorbed with no reflection at all.

Absorption is thought to be due to varying kinds and degrees of unsaturation of "chromophores" or "color-carrying" groups within molecules. Without going into detail, the essence of the idea is that molecules with certain open areas may possess "vibrating" electrons, that is electrons which may actually jump about in the molecule setting up a vibrating motion known as chemical resonance. If this resonance frequency corresponds to a wave length of light this wave portion of the incident light is absorbed

and the complementary color reflected.

Schemochromic color

The three principal structural colors are; whites, when total reflecttion occurs; Tyndall blues, resulting from scattered reflections through materials containing microscopic particles; and iridescent colors produced by interference effects on light reflected through thin layers. While other factors may sometimes be involved a consideration of these should be adequate to attempt to explain the colors of Bettas which involve schemochromes.

True pigments

Of the many biochromes only certain ones will be considered as probably involved in the production of phenotypic color of Bettas. These will be considered as separate classes.

Carotenoids The carotenoid pigments are naturally occurring, fat-soluble, nitrogen-free, yellow, orange, or red compounds, synthesized by plants but found in many animals. They were once known as lipochromes and are said to be the most widely distributed of the various conspicuous pigments by far. They and their derivatives are associated with many biochemical activities. Yellow pigments are sometimes referred to as Xanthins and are often mentioned in discussions of aquarium fish coloration. The term xanthophyl is used synonymously with lutein, both terms having been used for many years to describe pigmentation caused by certain carotenoids.

The carotenes are widely variable but are best known as the reddish-oranges, yellows and combinations of these. They may, however, also be purple, green, blue, gray and brown when in conjunction with various proteins. They are found throughout many tissues including skin, fats, liver, eyes, and familiar fish oils.

Fox classified animals as follows for pigmentation:

1. "Carotene animals" which selectively assimilate and store mostly hydrocarbon types of lipochrome

2. "Xanthophyll animals" which store only polyene alcohols and reject carotenes or possibly convert them into xanthophylls

3. "Non-carotenoid animals" which store little or none of the carotenoid pigments because of complete voidance or conversion by degradation

4. "Non-selective animals" which readily assimilate and store either of the two basic types of lipochrome mentioned

Carotenes, therefore, have alternative possibilities of treatment when introduced into a system. They may be eliminated unchanged via the feces, assimilated and stored in an unchanged state, degraded and consumed, or assimilated and converted into other carotenoids. The last possibility includes conversion into "fish xanthophylls" among other things. It should be mentioned that these are not mutually exclusive.

Chromolipids or lipofuscines These include some pigments which appear to be derivatives of fatty tissues. They are ordinarily yellowish but may also be brownish or even nearly black. There is little in the way of literature dealing with these pigments in fish and

none at all referring to Bettas.

Pterins Goodrich (1941) found the red color of Bettas to be erythropterin rather than a carotenoid or a melanin. Pterins are best known from the insects, particularly the Lepidoptera. According to Fox, they appear to be excretory products of some kind but are not always found in the excreta of animals that possess them. They occur in special cells termed erythrophores. Xanthopterin has been shown to function antagonistically to tyrosine-tyrosinase darkening systems, an effect which can be reversed with riboflavin.

Purines Guanine, one of the constituents of nucleic acids and usually considered to be a waste product, sometimes functions as a white pigment, though the effect may be at least partially schemochromic. Fish have developed systems whereby they deposit guanine in the form of crystalline particles in cells called guanophores, also referred to as iridophores or iridocytes. The substance may be present in dense masses in the skin, particularly the light underparts of fish. The metallic color of fish is ultimately produced by aggregations of these crystals viewed through various screens or with associated pigments so that a large number of variations is possible. An interesting animal structure involving deposition of guanine is the tapetum, a light concentrating structure located in the back of the eye (between the choroid and retinal layers) of animals such as cats which routinely use the visual sense in dim light. The tapetum reflects light as observed in the eyes of such animals at night.

Melanins Melanins and melanoids are well-known, widely dis-distributed pigments of a class known as indoles. They have been

studied extensively in many animals. The indole pigments are catabolic products of the amino acids tryptophan, tyrosine and phenylalanine, essential in man. Of one of the melanoids, Fox (p. 220) comments:

> The simplest indole pigment of the melanoid class is the 6-5-quinone-2,3-dihydroxyindole-2-carboxylic acid. This is a red compound, constituting an immediate link in the formation of melanin (Raper, 1927)

Since the suggestion has been made by Wallbrunn (1951) that red color in Bettas may also be attributed to unoxidized melanin, the possibility of confusion between melanin and pterins may be serious.

The melanins are of special interest in fishes because of the various functions they serve. They typically occur in cells known as melanophores, which themselves are variable. The configuration of these cells and the arrangement of pigment particles in them contributes to the over-all appearance of the organism, as do the number and distribution they have over the surface. The pigment distribution may be altered, as may the cell population itself, by various external and internal factors.

The internal factors operate by altering cell configuration or distribution of pigment in the cell. They are neural or hormonal in character and they operate by changing the pigment cells. When pigment is dispersed throughout the cells the organism appears dark. When it becomes concentrated the organism is light. Changes may be very rapid or quite slow and are related to the emotional and or physiological state of the individual. Of the possible external factors, light is probably the most influential. Darkness and lightness is usually

associated with a blending of the organism's color with its background. A medium-colored fish may be able to lighten when placed over a light background or deepen when placed over a darker one. As may be inferred, the neural and hormonal controls may bring about responses rapidly, even in seconds. Long-range shifts (several days to weeks) are due to changes in cell population because of disintegration of existing cells or growth of new cells, alterations of chromogen, enzymes, or other substances, brought about by dietary alteration or other external interaction.

Flavins These substances, which actually impart little to the overall pigmentation, show some interesting effects in some fishes. It was noted that they are stored mostly by the kinds of fish which have few or no scales, which probably limits them as significant contributors to Betta coloration. They are a pale yellow shifting occasionally to pale greenish or orange. They are stored, usually, as flavoproteins and may vary in quantity. In some species the eggs contain large amounts prior to fertilization but rapidly lose them following the process.

Of special interest is the coexistence of flavorproteins with melanins and carotenoids in the same pigment layer. They may be lacking in other layers which may be rich in quinones. There has been some speculation that the flavoprotein may be serving as an intermediary oxidant in melanogenesis, but the disclosure that riboflavin may inactivate tyrosinase and certain other enzymes in visible light suggests that the flavin may control rather than catalyze melanin formation.

Other pigments There are numerous other pigments, such as the

tetrapyrroles, porphyrins, bile pigments, haemocyanins and amino acid pigments, some produced by plants and rarely found in animals. Those discussed here are felt to include all which may be involved in a significant way in Betta coloration.

Color and Fish Genetics

Ginsburg (1929) observed developing pigment cells in guppies and suggested that guanophores appear first, followed by melanophores and finally xanthophores in developing fish. He also described the movement of "plaques" and melanin granules which he thought to be moving about independent of migrating cells. Becher (1929) observed that guanophores and melanophores were actually cells and that they contained motile granules or particles.

The inheritance of color and pattern variation has been studied extensively in certain aquarium fishes. Some of the more important studies will be described briefly, including those which have involved Bettas.

The work of Dr. Myron Gordon through a long series of studies dating back to 1927 with many co-workers has contributed in an outstanding way to our knowledge of genetics in fishes. His work was primarily with the Xiphophoran or Poeciliid fishes, small live-bearing (ovoviviparous) species from Mexico and Central America.

There are a number of species of the Xiphophoran fishes which are divided into two main groups characterized by the Swordtails and the Platyfish or Platies. Superficially they are rather distinct and were for many years considered to be separate genera. In wild populations hybridization

did not occur between the two main groups. The best studied species, Xiphophorus helleri and Xiphophorus maculatus were easily hybridized in aquarium stocks, however.

Wild Xiphophorus helleri is quite uniform but it was discovered that there were a number of color patterns in the wild populations of Xiphophorus maculatus. This polymorphism in the latter species has been studied extensively by many workers. A multiple allelic series of genes controlling black color patterns on the tail was discovered as well as a number of other color regulating genes. These have been of particular interest since their effects are modified in various ways in the hybrids. Many new color types have been produced in the laboratory and mutations of various kinds in either of the original forms or the hybrids have been transferred from one form to the other by breeders creating probably the largest assortment of variable types to be found in the aquarium fishes.

Gordon (1927) described two kinds of melanophores in these fish, small ones (micromelanophores) and large ones (macromelanophores). The micromelanophore development is controlled by an autosomal locus while the macromelanophore patterns are sex-linked. Several macromelanophore pattern genes were investigated and it was found that when one of these from Xiphophorus maculatus occurred in hybrids with Xiphophorus helleri a melanoma would develop. This result was attributed to "genic imbalance" in the hybrid.

These and other observations led Gordon (1953) to suggest that fishes were most useful as experimental organisms for the evaluation

of atypical pigment cell growth. The untimely death of Dr. Gordon in 1959 terminated a most productive career which opened some very important lines of research.

The work of Goodrich (1929) was a basic presentation of the state of genetic study of aquarium fishes at the time. He summarized the reported work with guppies, *Poecilia reticulata*; rice fish, *Oryzias latipes*; swordtails, *Xiphophorus maculatus*; goldfish, *Carassius auratus*; a killifish, *Rivulus urophthalmus*, and various hybrids, in the area of genetics and pigment patterns. Mention was also made of sex determination and fish chromosomes.

Goodrich (1933) described the light-colored recessive mutants of *Oryzias* which were shown by use of "dopa" reaction to possess the necessary oxidase of melanin formation and inferred that lack of a necessary chromogen (tyrosine) in melanoblasts probably was producing the mutant effect. The observation was made that, in a similar test with *Carassius auratus*, oxidase appeared to be lacking.

Later, Goodrich (1935) summarized pigment development in several species and related it to developmental processes involving the induction of chemo-differentiated cytoplasm, formation of chromogens and conversion of chromogen by oxidases, postulated to be under the control of at least three genes. This interpretation would probably be acceptable today if the term "gene" were replaced by a less confining one such as "biochemical system". The development of patterns involving different types of melanophores was compared in several species and related to genetic control. The fairly well known color development of *Carassius auratus* was briefly

reviewed. Young goldfish are typically dark colored because of integumentary melanin but they also possess reddish carotenoid pigments. They become "gold" when melanophores begin to disintegrate and disappear, unmasking the red-gold color. Sometimes, in variegated fish, for example, other pigment cells also disintegrated. While some research has been done in the area of pattern development, these studies are limited in fish. Goodrich (1935) discussed the development of certain color patterns in fishes such as the alternating light and dark stripes of the Zebra fish, Brachydanio rerio. He was unable to adequately explain such phenomena with information then available. Additional information has been provided since. Lehmans and Young (1959) gave experimental evidence concerning development of patterns in three salamander species. They found pigment cell origins to be the neural crest. Pigment cells differentiate and migrate at different times. Some types tend to aggregate with one another while repelling other types. Tissue influence seems to be minimal. They caution, however, that systems are extremely variable and that it would not be wise to assume identical patterns of development for all organisms.

More recently, Goodrich and others (1941) made chemical identification of pigments in some better known fish species. They found melanin and carotenoids, lutein (xanthophyll), zeaxanthin, violaxanthin, and the pterin, erythropterin. Their analysis showed a red from Betta splendens to be erythropterin and a yellow to be lutein. They mention the occurrence, in Xiphophoran fishes, of a peculiar and unique "xantho-erythrophore" which appeared to be a xanthophore in which erythropterin

is also produced.

The term albinism has been used by many fish workers to describe the absence of melanin, or more specifically dark pigment. The possession of a pink or red eye has been considered a prime requisite. A very red fish with a red eye may be called red albino, for example, while a white or colorless fish may not be considered albino if it has dark eyes.

Gordon (1952) found that Xiphophoran hybrids could be homozygous for the recessive "albino" gene and, if they also possessed macromelanophore genes, would develop typical "melanomas" with no visible melanin. These albinistic cells are referred to as "amelanotic" melanophores. The problem of pigment cell nomenclature was discussed, especially with reference to those bearing melanin. While technically any cell wich contains melanin may be called a melanophore a suggestion was adopted that normally, only cells which actually synthesize melanin be termed melanocytes, thus excluding such cells as macrophages which obtain their pigment by ingestion.

Atz (1962) presented an extensive summary of the effects of hybridization on pigmentation in the genus Xiphophorus which was, in part, an extension and confirmation of some of Gordon's work. All species have been hybridized with others though they do not cross in the wild. The effects of the numerous micro- and macromelanophore patterns in various hybrid genetic types have been studied extensively and reactions associated with phylogenetic relationships. Studies of "constellations" of modifiers in various genetic backgrounds have revealed similarities

between species in more sophisticated ways than by direct observation. The knowledge of pigment cell and pattern development and genetics in *Xiphophorus* is far advanced.

Genetic Studies of *Betta splendens*

The "Cambodia" mutation

The first mutant forms of *Betta splendens* to reach this country were those received by the previously mentioned Frank Locke of San Francisco. These fish were very light in color, with long fins. Apparently Locke did not recognize these fish as variants of *Betta splendens* but considered them to be new species. He termed the light colored fish *Betta cambodia*, a term which was soon shown to have no valid taxonomical basis but one which has stuck with this color variety to this day.

Essentially, the Cambodia type is albinistic. Black pigment is nearly absent over the body in most individuals though some produce a few visible groups of dark cells which may be quite noticeable on the light colored background. Aquarists do not refer to them as albinos, most likely because of their fully pigmented eyes. Goodrich and Mercer (1934) reported that Cambodia was a simple recessive trait and assigned the symbol *c* to it, as was conventional in animal color designation. The "dark" allele was labeled *C*. The findings were confirmed by Domantay (1935), Umrath (1939), Eberhardt (1941), and later by Wallbrunn (1958) and others. Variations in Cambodia fish are shown in Figure 4.

At this point it should be noted that Umrath used the symbol *c* to

designate what he referred to as "albino". It is obvious that he was differentiating between albino and Cambodia because he also included the following:

> Ein Zurücktreten der Melanophoren scheint bei Fischen vorzuliegen deren Rot und Gelb sehr stark in Erscheinung tritt. Diese roten Fische, die ich mit mm bezeichnen will, sind rezessive, die heterozygoten Mm gleichen phänotypisch den homozygoten MM. Für alle hier möglichen Kombinationen habe ich Zuchten, die den Erwartungen entsprechen.

This description fits Cambodia exactly but later workers have not referred either to this distinction by Umrath or the symbol designations he used! A "total albino" Betta was reported by Schreitmüller (1927) which was said to possess "rose-colored flesh", transparent so that internal organs were visible, and "red, bloodshot eyes". There have been a number of contemporary reports of albino Bettas but none are substantiated and no genetic investigations have revealed anything nor have symbols been applied.

Veil-tailed Bettas

The abnormal extension of finnage in many aquarium species has come to be identified almost universally as "Veil-tail", or "veiled". As might be expected, long-tailed forms rapidly became extremely popular with breeders. Eberhardt (1941) is the only one to have attempted a genetic analysis of this character and suggested the symbol P for the dominant long-finned form versus p for the normal, short-finned condition. Gordon (1954), however overlooked this study and stated: "All attempts to

Figure 4. Bettas of the "Cambodia" type. All are represented by the genetic symbol cc. The numerous variations are due to the iridocyte color each possesses

a. Male with a medium density of iridocyte color of the type vv (green) and Ri-

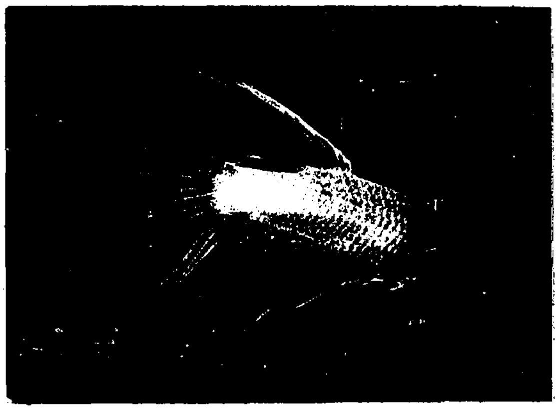
b. Male with some melanophores present, producing a general darkening of the phenotype and a more obvious expression of the same green color

c. Female of the intermediate blue type designated Vv. This individual has the normal distribution and intensity of iridocyte color

d. Male of the same type as the female with the more extended iridocyte pattern Ri-

e. Male of the homozygous blue type VV. Cambodia with normal distribution of iridocytes

f. Male with the extended iridocyte distribution of Ri-. This fish also shows an extension of red pigment which would not be expected in "normal" Cambodias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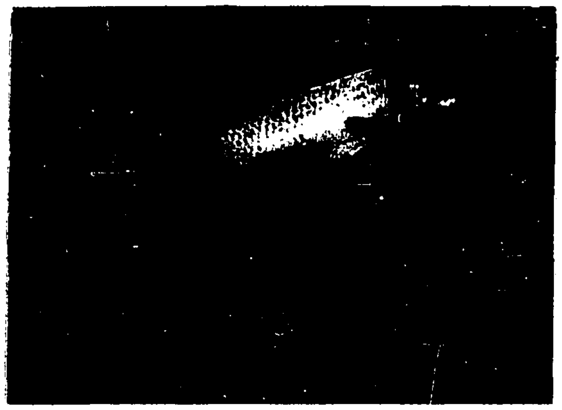
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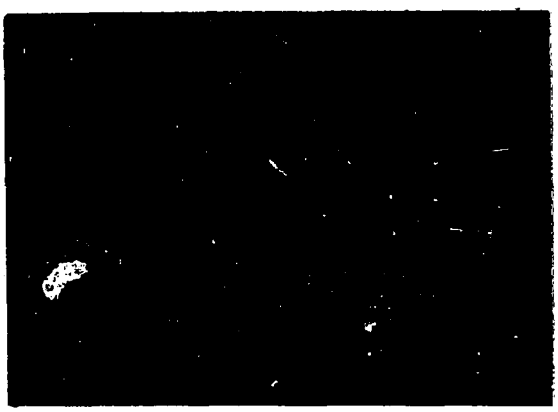
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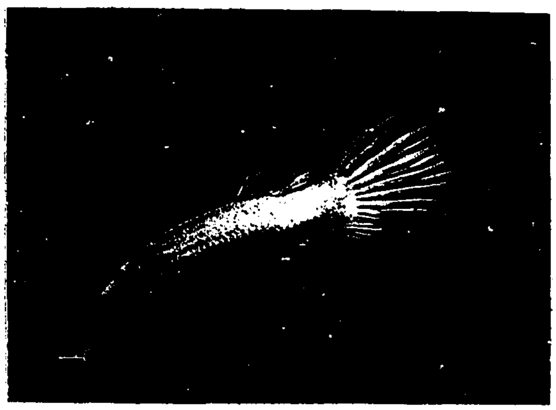
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determine whether the short-tailed trait is dominant over the long form or vice versa have been inconclusive".

"Blond" Cambodia ("bright")

Wallbrünn (1958) presented evidence for a second genetic mutant which reduces dark pigment. The effect is not as severe as in Cambodia but is quite evident to the practiced observer. He was interested in red color varieties and found that this reduction altered the appearance of red. His symbolic designation was b to signify bright red color, which prevailed when the fish possessed this light variation. By counting melanophores in a "deep" zone of a scale area fish could be grouped as having 0-15 cells per zone to 266-330. While there were fish in all groups throughout the range, it was possible to identify the two types visually and the cell counts of b fish did not exceed the 78-93 range. Umrath had attempted to describe a variation which may possibly be the same. He described fish which had "ein prächtiges, sattes Rot" (a splendid, deep red), which he symbolized l, apparently since he considered the variation to be due to a reduction in lipophores which allowed more red to show. On the basis of limited progeny (a total of 7 of 41 fish were ll) he concluded that the variation was recessive. He suggested that his gene mm would appear red in these fish. Fish of the normal, light, and Cambodia types are shown in Figure 5.

Wallbrunn's data confirmed the recessive nature of the mutation and his symbol b seems logical, though it refers to the final appearance of

Figure 5. Variations in darkness or lightness due to the presence of varying amounts of melanin in normal, Wallbrunn's "bright" (called "blond" by Gordon), and Cambodia

a. A _bb_ fish and a normal. The dorsal surface of the head region and the cheek area are where this color variation is best observed

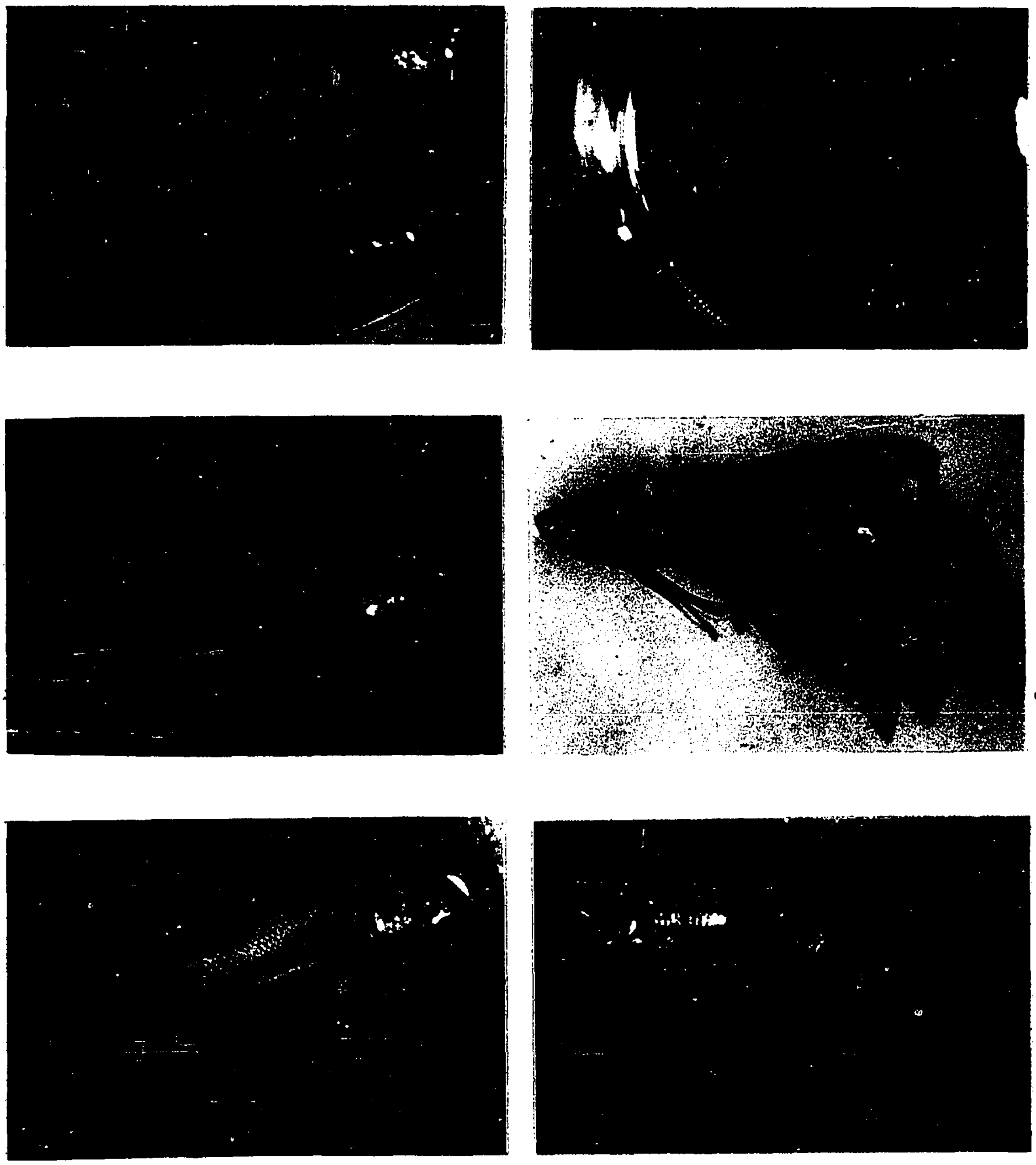
b. Dorsal view of the three types. The left fish is _bb_, the center one _cc_ and the right one, normal

c. A "yellow" Betta. A Cambodia mutant with other color variations. Note the dark eyes

d. A normally dark fish. The cheek area, particularly, is dark

e. A Cambodia mutant. This fish has considerable amounts of guanine deposited in its skin. Note pigment of the iris of the eye

f. A _bb_ fish. Note the light color of the dorsal head region and the cheek and chin area



a b c d e f

red rather than something it itself has an effect upon. Gordon (1954) then confused the issue by comparing the mutant with the light mutation in guppies called blond. The comparison was reasonable but the interpretation of Wallbrunn's symbol _b_ as blond may not have been.

"Bald" Bettas

Eberhardt (1941) described still another mutant involving a modification of the melanophores. It appears that these fish developed a light pattern on the dorsal surface of the head and anterior body which, in the proper genotype, is quite obvious in three-month-old fish but which may be obscured to some degree later as melanophores populate or repopulate the area. The character was genetic and inherited as a simple recessive. Stocks were not preserved, however. Gordon (1954) referred to it as a "bald" spot though Eberhardt called it "dorsum lucidum" and used the symbol _dl_. Wild Bettas have a light medial stripe visible from the dorsal aspect but it does not resemble Eberhardt's illustration.

Color produced by guanophores or iridocytes

Variations in the metallic sheen of Bettas were described by Goodrich and Mercer (1934). They mentioned:

> Highly colored fish known to fanciers as _Betta splendens_; the blue Betta, _B. cyana_; the green Betta, _B. smaragdgreen_; the red Betta, _B. rubra_; and a light blue Betta.

No experimental data were given involving the inheritance. Umrath

(1939) presented limited data (15 and 8 to represent a 1:1 ratio for example) but did use the terms "Grünen", "Blaue", and "Stahlblau" to describe the phenotypes. He did not include symbols or literature references. Eberhardt (1941), and later Wallbrunn (1958) confirmed the inheritance of three phenotypes developing from the two alleles at a single locus.

One allele was designated v (viridis) by Eberhardt (1941), and G (guanophore) by Wallbrunn (1948) who, because of World War II, was unaware of Eberhardt's work at the time. Both workers concluded that one homozygote (VV or GG) produced a dull blue phenotype known as Steel blue. The heterozygotes (Vv or Gg) are intermediate or "bright" blue, and the other homozygotes (vv or gg) are green.

An additional variation involving iridocyte color is its density and distribution. Eberhardt (1941) designated an allele ri (reduzierte Iridocyten) and an alternative Ri, inherited as a dominant, which was characterized by marked extension of iridocyte colors. Wallbrunn (1948) postulated two possible genes effecting this color, a and s. The a locus was thought to control iridocyte color spread on fins while the s locus did the same to the body of the fish. Wallbrunn later seems to have accepted the priority of terminology of Eberhardt as no further mention was made of the two-locus theory. Examples of the variation produced by iridocytes are shown in Figure 6.

Figure 6. Various phenotypes produced by genotypic combinations of iridocyte color

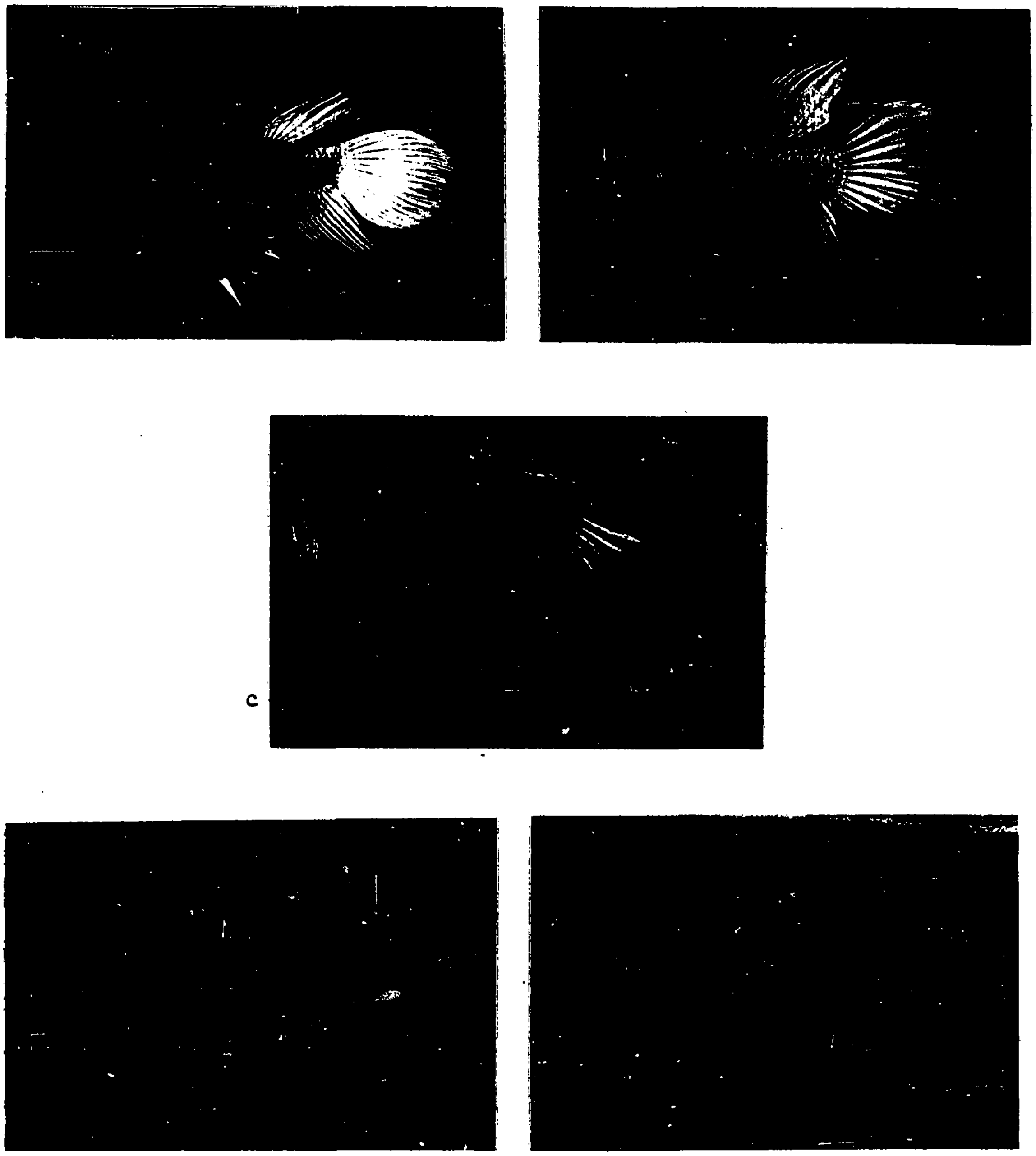
a. A high sheen steel blue male of the designated genotype VV, Ri-

b. A similar male of the designated genotype vv, Ri-

c. A similar male of the designated genotype Vv, Ri-

d. A green male with more extensive distribution of sheen than expected in wild types but not as extreme as that of c. This fish would still be classified vv, Ri-

e. A steel blue male which would be classified VV, riri. The limited spread of iridocyte color is similar to the wild type condition



a b c d e

Other characteristics

The only other abnormality or variation investigated and formally reported is a reduction or absence of the ventral (pelvic) fins, a not uncommon condition. Wallbrunn (1951) described this and mentioned abnormalities in fin ray counts which seemed to be associated with it but came to the conclusion that the effect was non-genetic.

Wallbrunn (1951) pointed out that there are two characters which seem to be affected by sex. These are fin length and the development of red color on the body. Females may get slightly elongated fins if of the domestic type but rarely enough to be accurately distinguished from normal. Males of the long-finned type are easily distinguished because of the continuous extensive fin growth. Most pigments appear equally on both sexes but red color is exceptional. The female's body shows at most a limited amount of red, though the red of the fins may be as intense as that on males.

A number of new variations exist which have had limited study, if any. The only mention of any of them has been through popular hobby publications and aquarium club bulletins. Colorless, yellow, red, brown-black, variegated and piebald, and double-tailed forms are now available.

Sex determination in Bettas

It is the general opinion of breeders that something unusual is involved in sex determination of Bettas. Inconsistent sex ratios in my stocks have been striking.

Chromosomal studies of the Betta have not revealed satisfactorily whether real sex chromosomes exist. Bennington (1936) felt that it was possible to distinguish them but Svaerdson and Wickbom (1942) did not agree and no further investigations have been reported. They did agree that the diploid chromosome number is 42. The chromosomes are all very small, short to punctate, and require further investigation before this aspect can be resolved.

Sex reversal has been noted and studied on a limited basis in Bettas. H. Schmidt (1930) obtained an approximately normal sex ratio from a cross between a spontaneously sex-reversed "male" and a normal female. Noble and Kumpf (1937) found a "male" which had functional testicular tissue which developed from the gonadal remnant of an ovariectomized female. A spawn obtained from this "male" produced 9 males and 12 females, again a reasonable representation of a normal sex ratio. E. Schmidt (1962) reported fertility in ovariectomized transformed "males". From three spawnings, one produced no hatching fry and was concluded to be infertile, and one produced six fry, the sex of which were not reported. The third produced 102 fry which were classified as all females at eight weeks. He concluded that this confirmed the existence of male heterogamety in Bettas. He made no mention of previous studies. Parent fish were taken to the University at Hamburg (Germany) where histological examination confirmed the development of functional testicular tissue growing in place of the lost ovaries.

The following statement by Schmidt is of interest:

> "With these results I went to the University, where I visited Professor Klatt, of Hamburg. He did not place much belief in my experiments when he heard about them, assuming that aquarists usually did not work carefully and were apt to let their enthusiasm lead them to false conclusions.....the examination proved that it is possible for a spayed female Betta to make a complete sex reversal".

This article appeared in a commercial aquarium hobby magazine and if a response has been made to it I am unaware of it. At any rate, I share Professor Klatt's doubt of at least one element of this report, the statement that "Eight weeks later I could determine exactly the sex of all the youngsters...". I have never been able to feel certain of sex classifications of Bettas at that age and no other investigator has expressed this confidence. In my experience, only the more precocious males are then obvious while all others retain the undifferentiated, female-like appearance.

In spite of the limited and apparently contradictory data concerning Bettas, the fact emerges that Bettas pass through undifferentiated stages during which time they might be considered latent hermaphrodites. They have not been convincingly demonstrated to have either male or female heterogamety on either a cytological or genetic basis. Finally, they exhibit extreme variation in sex ratios following maturation.

The factors participating in deciding the sex ratios have not been understood. Two previous scientific reports are available regarding the abnormal sex ratios found in Bettas. The first was that of Eberhardt (1943b) who investigated some aspects of the problem. He provided a

tabulation of sixty-nine spawns which, when classified as to percentage of females were found to be distributed from 0% to 100% and while there were more spawns representing approximately equal numbers of each sex, there were also many which deviated widely.

Wallbrunn (1951) provided data from his own experiences and compared his with Eberhardt's. His findings were in approximate agreement, that is, there was a rather general distribution throughout but also some extremes of all male or all female spawns.

In comparing these two sets of data some factors are of interest. There did seem to be a central tendency, especially in Eberhardt's work. More spawns contained 40-60% males than any other. Wallbrunn's findings showed this tendency to a lesser extent but this may be partly explained by the fact that he had only half as many spawns included. It was also shown, in each of the two investigations and when the results were grouped, that there was a definite inclination to the higher male percentages. Eberhardt found 45 spawns with more males, 24 with more females. Wallbrunn reported 25 spawns with more males against only 10 containing more females. Collectively this tabulated 70 with more males against 34 with more females, a highly significant deviation from equality.

General sex determination

In view of the uncertain status of sex ratios in the Betta, I have made a survey of sex determination information available in the literature. An excellent recent review of sex determination is that of Bacci

(1965). It presents examples of known possibilities for variation as far as sex-determining methods are concerned, along with several theories of a general nature regarding systems of sex determination, mechanisms and conditions which influence sex, and the possible evolutionary significance of some of them.

Reproductive systems may be classified in a variety of ways. Basically, it may be observed that organisms may reproduce by sexual or asexual means. If they reproduce sexually, they may be further sub-classed into organisms which normally have stable, permanent differentiation of separate sexes, called gonochorists, or into a heterogeneous class having some system other than normal gonochorism. Large numbers of the more primitive organisms fall somewhere in this latter category. Some fishes and some other more primitive vertebrates also fall into the second group.

Non-gonochorists can conveniently be sub-classified into categories of different degrees. Some are highly specialized and may be true hermaphrodites of one kind or another. Many are "false gonochorists", that is, an individual may function as one sex and then the other but at different times. Some function first as males, later as females and are called protandrous hermaphrodites. Others function first as females and are known as protogynous hermaphrodites. Still others do not have such regular functional shifts. Many have environmentally induced variations.

Bacci's monograph contains many examples of the various modes of reproduction. A selection of examples, representing a number of different phylogenetic groups, is presented here.

Platyhelminthes

It was determined that the parasite Schistosoma mansoni was able to differentiate from functional male to female. In limited infestation where no females might have been introduced some males would have degeneration of testicular tissue, and development and maturation of ovarian tissue.

Nemethelminthes

The weight of sheer numbers seems to influence development to the male side in Paramermis contorta, a nematode parasitic in Chironomus larvae. The more individuals that are present, the more of them that are stimulated to become males.

Mollusca

A Mediterranean limpet, Patella, has been discovered to develop from male to female following the breeding season. There are some "pure" males and females, some that have relatively longer male or female stages and others which exhibit the "normal" condition for the species of male, intermediate stage, female.

In the gastropod, Calyptraea, the organisms change sex from male to female between the first and second year. There is a relationship between developmental size and development of the secondary phenotypic sex, smaller individuals typically being males, larger ones females.

A gastropod "pest" found in oyster beds, Crepidula fornicata, may occur in piles or associations of single organisms or they may be isolated. Isolation tends to stimulate individuals to become females, while in aggregations, the larger individuals in the centers of the piles are usually females while the outer smaller individuals are generally males. It appears that the presence of some females stimulates newer organisms joining the aggregation to become males.

Echiuroidea

The well-known case of *Bonellia viridis* indicates that the proboscis of the female may elaborate a masculinizing hormone which stimulates undifferentiated larvae to become males. Larvae coming into contact with a female become associated with the proboscis and exist as a rudimentarily developed, "parasite" of the female. Free larvae tend to develop into females. In addition to the unusual system of establishing the phenotypic sex, several constituents of the water have been shown to have a modifying effect upon the phenotype. HCl, CO_2, K, and traces of Cu were found to stimulate production of up to 92% males, while removal of SO_4, and Mg induces the development of up to 90% females.

Annelida

The polychaete worm, *Ophryptrocha puerilis* is protandrous, passing first through a male stage, followed by a stage in which it is a morphological hermaphrodite, then it becomes a female and finally it ages and shifts back to the male side. The development was found to coincide with growth in the sense that the addition of segments seems to be of prime importance. The individuals having low numbers are typically males, whereas if the segment number passed 20 or so it shifted and became a female. It was discovered that this general pattern could be modified or countered by altering various environmental elements. Hunger had a masculinizing effect as did increased K^+ ions in the water.

Crustacea

The larvae of a parasitic marine copepod, *Ione thoracica*, attaches to the gill of another crustacean, and the first larva which becomes attached invariably becomes a female. Additional larvae are induced then to become males. The system insures the presence of both sexes in cases where numbers are very low.

Teleostei

> A Mediterranean species, Coris julis, which was considered at one time to be two species, is in reality one in which individuals are usually of one morphological type and female, later to become males of the other morphological type. However, some of the first type are males, some of the second females, and transitional types are known. It appears that protogynous hermaphroditism is widespread in the Labrid family to which Coris julis belongs.

In his summary of this material, Bacci considered the genetic balance system proposed by Witschi and Goldschmidt. Witschi (1957) reviewed concepts developed to that time related to balance and induction. He suggested that Hertwig's work with Rana esculenta proved individuals do not differ by presence or absence of single male or female determining genes but by quantitative balance of many. Goldschmidt's Lymantria formula was adapted for the case of sex races of frogs by the addition of a minor female-determining factor to the Y chromosome. If the sum of all female "quanta" exceeds that of the male genes, the individual is a genetic female. The sexes are referred to as epistatic and hypostatic and development under normal conditions allows the epistatic sex to prevail, though conditions could arise whereby the hypostatic sex can, at least temporarily, manifest itself. Environmental factors, such as delayed fertilization or temperature extremes thus can cause reversal.

Witschi emphasized the medullary and cortical location of developing gonia, and the fact that their development seems to depend upon the location in which they developed rather than their genetic make-up. The gonadal cortex induces female development and the medulla induces male.

He further noted that the terms cortex and medulla are properly applied to the gonads of amphibians and higher vertebrates and cited D'Ancona (1956) as indicating they are not proper for teleosts, cyclostomes and invertebrates.

Disregarding exceptional organisms, Witschi's system suggests that sex genes may control cortical and medullary development, and this might be considered their only function. The cortex or medulla may in turn acquire inductive properties. External factors seem to operate by interfering with inductors, certainly not by altering genetic constitution. If the inductors are inhibited, the hypostatic sex can develop. Witschi also referred to inductive antagonism. The right rudimentary medulla of the female chick, sex modification in male-female parabiotic salamanders, and the "free-martin" female in cattle twins are examples of what he considers repression by antagonism.

Finally, he postulated chemical substances, corticin and medullarin, as being involved but suggests that they are probably proteins of some kind rather than steroid hormones. In lower anurans and salamanders, particularly, almost all of the steroids, androgen or gynogen, had feminizing effects. In most ordinary frogs they had the opposite, a masculinizing effect.

From the lengthy discussions by Bacci of environmental factors which can affect sex ratios, the following summary is gleaned:

Inorganic compounds

The *Bonellia* case mentioned earlier and the case of *Tigriopus japonicus*, a marine copepod described as a strict gonochorist, are examples of organisms known to exhibit sex ratio shifts due to various environmental changes. The presence of substances (di-iodotyrosine, chloretone, chloral hydrate, KCl, $MgCl_2$, $MgSO_4$, NaCl, and LiCl) or altered temperatures (rises for example) stimulated growth rate. Accelerated growth rates produced more males. Substances which retarded growth also increased, even though possibly indirectly, the number of females.

Amputation

The removal of gonadal tissue, ovary for example, may result in the growth of other tissue, if rudiments are present. Several examples have already been mentioned, including oysters, chickens and some teleost fishes.

Hunger

This was shown to stimulate ratio shifts toward the male side in oysters and copepods. It would appear to be an effect opposite to the growth rate factor of *Tigriopus*.

Crowding

This factor has been demonstrated to have the effect of extending the male phase in oysters though the reason was not established.

Temperature changes

Temperature has been demonstrated to be intimately involved in final sex ratio determination in some species of the crustacean Gammarus In *G. duebeni* it was disclosed that temperatures below

5°C produce excessive males. Temperatures above 6°C show an abrupt reversal to excessive females. In amphibians extremely high temperature causes destruction of gonadal cortex and low temperatures interfere with gonadal medullary development, either of which might have an affect on the sex ratio.

Hormones

Examples have already been given at various times of various effects produced by hormones. Again, the Bonellia case may serve as an example where a substance elaborated by one individual may influence the sex development of another.

These are only a sample of the many known factors which in some way influence sex. It is felt that they include most of the factors which may have something to do with sex determination in Bettas.

Sex Determination and Sex Ratios in Fish

Peculiarities of sex ratios in many fishes are known, and though some of the cases are confusing, they are not necessarily mysterious. The problem may be properly related to that of basic sex determining systems, hermaphroditism, and sex reversal.

Various types of hermaphroditism in teleosts were summarized by Eggbert (1933), at least as well as they could be at the time. It was known that both male and female gonads were found in individuals of certain species but also that there were hermaphroditic gonads. Mention was made of Xiphophorus and another genus, Periophthalmus (mudskippers) which, during sex differentiation, passed through what was termed "phases of temporary intersexuality", during which sex could be influenced environmentally. Sex-reversal in Xiphophorus helleri was discussed and the

author was of the opinion that sex-reversal may have been under the influence of the "rest-body" which could inhibit the action of female hormones. These "rest-bodies", which had been found in various tissues, were observed by many investigators in gonadal tissue and were first considered to be atretic eggs. They were later determined to be cysts of a phycomycetic parasite, Ichthyophonus hoferi, which apparently does have some relationship to arrhenoidy which is not altogether clear.

Friess (1933) also considered the problem of sex reversal in Xiphophorus and described in detail the anatomy and histology of the generative organs both in normal and sex-reversal cases. He considered the variation in sex ratios from generation to generation and the restoration of normal ratios a few generations after introduction of a sex-reversed individual to be incompatible with the operation of a sex chromosome system of the conventional sort and suggested the existence of some form of balanced hermaphroditism.

Kosswig (1933) suggested that certain color-factor loci were also female-determining loci in the swordtail Xiphophorus helleri basing this hypothesis on previous reports by others that Xiphophorus pass through "protogynic" stages during development, when they are of female tendency. A number of fishes, including Bettas, go through undifferentiated immature stages where they resemble females but there is definite morphological differentiation to male or female upon maturity.

Winge (1934), after a number of years of work with Poecilia reticulata, postulated that sex determining genes were distributed throughout the genome, some being female determining and others male. A net

superiority of one kind would produce males, the other, females. He thought that the factors in the sex chromosomes had a primary and stronger effect than autosomal sex determining genes but that it might be possible to accumulate enough of one or the other kind to over-ride the primary determiners. Another factor was that an individual might be nearly balanced so that it would be only a weakly determined male or female, or, on the other hand, it might have accumulated large numbers of one kind and then would be a strong male or female.

Extending this idea, Winge was apparently able to identify strong and weak strains and to produce strains having altered sex-determining systems; that is, he was able to produce XX males and XY females, reversing the normally male heterogametic condition. The occurrence of a number of sex-linked color markers on both the X and Y chromosomes, along with the disclosure that linkage evidence from crossing over suggested no map distances greater than around 10 units and indicated that the sex chromosomes, if such there were, were largely homologous and small. The inference, obviously, is that the X and Y chromosomes are differentiated at a primitive level if at all. In an evolutionary sense, they may merely be chromosomes with a few more sex-determining loci than others.

Gordon (1952), summarizing his work and that of others, reported that in Xiphophorus maculatus there had been found wild populations (collected from different rivers) with male heterogamety (XY system) and others with female heterogamety (termed by Gordon WY with YY males). These were demonstrated to be the same species by a variety of methods including various color markers, general morphology and the free

hybridization of the two types. The hybridization experiments showed progeny counts consistent with this theory and crosses between XY males from one race and WY females from the other produced WY and WX females and XY and YY males in expected proportions. Gordon disagreed with Winge's idea that the XX-XY system was evolutionarily ancestral in these fish and in guppies and that the WY-YY system had arisen from it. He felt that it was more likely that the two systems had developed independently and that there was no satisfactory explanation for the development of both systems to this point.

The Medaka or Japanese Rice Fish, *Oryzias latipes*, has been of genetical interest for many years. Color variations have been investigated by Goodrich (1929) and Goodrich, Hill and Arrick (1941), among numerous others. The sex-determining system is XY with male heterogamety. In recent years, Yamamoto has conducted a number of investigations dealing with hormone effects on sex determination and experimental alteration of phenotypic sex in this species. These are discussed later, in connection with intersexuality.

Kallmann (1962) described gynogenesis in *Poecilia* (*Mollienisia*) *formosa*, a species closely related to *Xiphophorus* and *Poecilia reticulata*. Wild populations are overwhelmingly female with only three or four males having been found. The females mate with other species of the genera but apparently do not have real amphimixis or a reduction division. The descendants constitute a clone, a fact which was demonstrated by a tissue transplant-histocompatibility test. Two major and one minor clones were identified in the wild populations studied and the population structure

remained relatively constant over a nine year period.

The histocompatibility test was also used to study relationships between individuals taken from wild populations of the killifish Rivulus marmoratus. Groups of the organisms actually constituted "clones" and individuals could be relegated to proper clones by this method. Kallman states that they have ovotestes and self fertilize!

A monograph on intersexuality of vertebrates, including man, edited by Armstrong and Marshall (1964) includes a most informative section on fishes by J. W. Atz. It may be summarized very briefly by stating that there are fish groups at various phylogenetic levels which include normal species exhibiting every conceivable variation of sex determination.

Atz indicated that many species are normal gonochorists, having separate sexes and reproducing in various ways but with no question of change in sex. Others are not so nicely organized. The term "intersexuality" is used to describe the many species which do not behave so precisely. These are intersexual in morphology or some other feature, especially involving reproductive phenomena.

The organisms of interest, since they offer clues to the problem of Betta sex ratios, are those which show variation in some way related to intersexuality. In aquarium fishes there are many that are egg layers, others that are livebearers. Both groups contain species which go through stages where sexes are morphologically indistinguishable. Many go through these stages but upon reaching sexual maturity undergo significant dimorphic change so that normal individuals are neither anatomically nor functionally able to perform as the opposite sex.

The situations of interest are those in which individuals may be altered, by whatever possible means, to function as the opposite of the "genetic" sex. This requires undifferentiated stages with bipotentiality or latent bipotentiality in differentiated forms. This requirement suggests a kinship to intersexuality and hermaphroditism. Examples of some of the best known of these are presented in summary here.

A number of examples of arrhenoidy (masculinized females) have been recorded from the guppy, Poecilia reticulata, as have others of what appeared to be parthenogenesis. Some females which produced fatherless broods were found to have an ovotestis but others did not. Individuals of this type produce progeny which appear to be genetic females on the basis of progeny test but some of these can become functional males (bearing out, it would seem, Winge's idea) which in turn, father all-female broods. Atz refers to this as abnormal synchronous hermaphroditism (the organism fertilizes itself, being able to produce functional gametes of both types at the same time). Gordon (1955) describes a method whereby guppies have been treated with methyltestosterone which has a masculinizing affect on the secondary characteristics of both immature and mature females. In these cases sterility may result but not functional reversal.

Atz discussed the causes of arrhenoidy in the Swordtail and other aquarium fishes and considered the views of many researchers. He concluded "We do not believe that functional sex reversal is possible in the adult Swordtail, or any other adult poeciliid for that matter", but also said "A wide variety of conditions or agents appears to be able

to initiate masculinization in female Swordtails, including old age, parasitic desease, X-rays, incomplete hypophysectomy and various hormones". This indicates that there is a general tendency towards masculinization and that anything which tends to interfere with ovarian function will result in masculinization. Moreover, he states that with the exception of the very few protandrous hermaphrodites, there has been no report in fishes of transformation of functional, adult males to functional, adult females.

The experiments of Yamamoto are reviewed briefly by Atz, including those involving successful reversal of both sexes in Oryzias with ample genetic evidence but these have been achieved by treating immature individuals with steroid hormones. One of Yamamoto's recent reports (1962) compares hormone effectiveness. Estradiol and stilbestrol reversed young males with around 3 to 3.5 times the effectiveness of estrone. Methyl testosterone has a strong masculinizing influence on young females as do, with lesser effect, testosterone propionate and androsterone. Other steroids, including progesterone, had no effect on either sex. Finally, it developed that there is a critical period during which reversal can be induced. After a certain stage of maturation, treatments were uneffective. It was suggested that further investigation was needed to determine whether fish hormones were all similar to mammalian or were of different kinds.

A common belief among Betta breeders is that the age of the parental stock influences the obtained sex ratio. Hannah (1955) found, in Drosophila melanogaster, a strain in which an increase in gynanders and a

reduction in females were obtained which could be positively correlated with aging of the parental female. Opposite to this result, Novitski and others (1953, 1956, 1958), in studies of human families, reported a positive relationship between the sex ratio and birth order, along with the age of the male parent. No similar positive correlation could be made in the case of female ages. Finally, in the March 1966 issue of the German periodical Datz, an aquarist reported that equal sex ratios were most likely to be obtained if the parents were of nearly equal age. He stated that "middle-aged" parents would produce a preponderance of their own sex if their mates were either younger or older.

Many other possible factors have been suggested as important in control of sex ratio in the Betta, but without much logical or experimental support. It appears, then, that there can be many factors to consider in the approach to modernization of Betta genetics. It is with these in mind that this study has been made and in relation to them that this report is given.

MATERIALS AND METHODS

Sources of Fish:

Domesticated types of Betta splendens were obtained from various aquarium shops and from certain experienced breeders, especially Mr. Walt Maurus of Livonia, Michigan, and Mrs. Dolores Bialk of Waukesha, Wisconsin. A number of other persons mostly from north central and northeastern parts of the United States, also contributed fish. No significance is attached to this geographical fact other than that there tend to be more fish keepers where there are more people.

I hoped to obtain wild Bettas to use as a normal reference for various abnormal types. Unfortunately, during the major portion of the research these were not obtainable. Some fish were obtained in 1965 from Dr. Walter Foersch, of Munich, West Germany, which were from stocks originating in Bangkok. These were stricken with a severe infection and were lost after only three spawns had been achieved. In the spring of 1968 a number of specimens were obtained by my brother, Major Dale A. Lucas, who purchased them from some Vietnamese boys whom he saw catching them near Tra Cu Special Forces camp about 30 miles west of Saigon, South Vietnam. He shipped 23 of these fish to me and they have thrived in my aquaria.

In lieu of wild stock a standard type for color comparison was derived from domestic forms available. This was achieved early in the investigations and was based primarily on the literature descriptions. When wild specimens were obtained they conformed well to the synthetic

standard though certain elements will require additional discussion.

Propagation of Fish

Housing and care

All breeders or potential breeders were kept individually in glass containers, usually one-gallon commercial wide-mouthed jars. These were filled to within two or three inches of the top with tap water which was allowed to stand at least 24 hours before use. Each jar was marked so that a fish in it could be identified as to which spawn it came from and, in addition, with an individual number if to be used as a breeder. For example, the designation 121-7 would indicate that it was the spawn #121 and the seventh fish to be used in a mating.

Matings were set up in glass rectangular aquaria and young fish were separated in smaller jars before finally being moved to the larger permanent gallon jars.

Water in which fish were kept was treated tap water from the Des Moines, Iowa, water treatment plant; its composition will be discussed later. Generally, the various chemical treatments and softening of water have been beneficial to aquarium fishes. Tanks were equipped with undergravel type plastic commercial filters when filtration was used. Jars did not have filters but water was changed every two to four weeks depending upon its condition.

Lighting was provided by fluorescent tubes designated white or daylight type. Lights were mounted in ceiling fixtures. Light duration was

not automatically controlled but typically lasted sixteen to eighteen hours a day. Heat was provided by a thermostatically controlled, gas-fueled space heater set to maintain water temperature around 80°F, unless the fish concerned were to be in a temperature experiment. Such experiments were conducted under special conditions.

Feeding

During the course of the investigations the fish were fed newly hatched brine shrimp (*Artemia salina*) larvae (nauplii) when they were small and a mixture of these and frozen adult shrimp when they were larger. Frozen adult brine shrimp were obtained from pet shops and nauplii were "hatched" from "eggs" (encysted forms) which are also available commercially. The fish do somewhat better with a more varied diet but they always seem able to reproduce and the bothersome problem of fin degeneration due to bacterial and fungal infections, related apparently to the decomposition of uneaten foods in the water, was minimal on the *Artemia* diet.

Artemia nauplii were "hatched" in an aerated artificial sea-water mixture in large glass funnels held in a wooden rack and plugged at the bottom with rubber stoppers though which a plastic airline was passed. Approximately 1 liter of water was used in each funnel into which was placed approximately a level teaspoon of the dry cysts. Vigorous aeration was continued for 48 hours at which time most nauplii would have emerged. They were then strained through a fine mesh cloth, rinsed in fresh water, and distributed to the fish with a plastic syringe-type kitchen baster.

Small fish received only the nauplii while larger fish got both nauplii, if surplus remained after feeding small fish, and the frozen larger shrimp.

Breeding

Matings were set up in aquaria of 12 to 15-gallon capacity. These measure approximately 12 by 20 inches. The tanks were filled to about 5 inches deep so that spawnings actually occurred in about 6 gallons of water. Tanks which were used for experimental matings were cleaned first with soap and water which contained a small amount of household bleach. They were well rinsed and filled with new water which was allowed to "age" at least 24 hours before fish were introduced. Spawning tanks were provided with commercial aquarium heaters which are thermostatically controlled and which kept the water within $1^{o}F$ of desired temperature. These were placed in tall jars of water inside the spawning tanks so that the shallow water would not cause excess heating of heater tubes with resulting breakage. No filter, gravel, or plants were used in spawning tanks. A glass cover was placed over all but a small area of the tank and a mark was made on the upright frame to indicate water level. Evaporation and temperature variation were minimized. Small amounts of water were added periodically to maintain the level.

Often the stimulus of new water will encourage a male Betta to build a bubble nest but it was discovered that an even more reliable stimulus was the presence of floating objects of the approximate size and color of a bubble nest or thin leaf. Styrofoam cups cut in half lengthwise and floated concave side down almost always stimulated males to build nests

under them.

Normal, healthy fish seem ready to spawn at almost any time. Females also breed at any time of the year in captivity but must naturally be "ripe", that is, they must have their ovaries filled with properly matured eggs.

One male fish was introduced into the spawning tank. A female was also introduced but confined to a quart jar so that she was effectively isolated from the male. Shields were placed so the fish could not see others in nearby tanks. The isolation procedure is routinely used by breeders of these fish so that the male's attention is directed toward the female. Her isolation is generally necessary until his preliminary aggressive behavior is converted to mating behavior.

Under the experimental conditions, the female would be released if the male had built a nest within two or three days. If this did not occur the mating was reset, with one or both of the fish replaced if they did not appear to be in spawning condition. If a spawning did not occur after several days and two or three attempts the tank was cleaned and reset and the process repeated.

The normal spawning behavior involves a period of display activity where both fish show intensification of coloration, gill and fin spreading and swimming behavior that is characteristic. The male will usually build a bubble nest within hours. When the female is released he will drive her to the nest. He "embraces" the female by wrapping his body around hers while she turns on her side and often finally on her back and releases a number of eggs. Both fish sink through the water in a trance-like state

for several seconds. Upon coming out of this they may both pick up eggs and blow them into the nest. Typically, however, it is the male.

For most spawns fry could be reared together for several weeks until they showed signs of crowding or young males began to show aggressive tendencies against their siblings. For various reasons a system was finally put into operation whereby a sample of fifty individuals was removed from the spawn. These were placed individually into quart sized glass jars seven to ten days after they had reached the free-swimming stage. This procedure eliminated the possibility of differential mortality due to some possible competitive disadvantage of one type or sex.

Fry were fed daily and grew normally to sexable size in 8 to 12 weeks. During this period water was changed two or three times. Though young fish show no sex differences it is possible to sex them quite accurately as they become sexually mature since the fins of domestic males grow longer, the color of the males deepens and they usually begin to make small bubble nests in their jars. With practice, it is possible to recognize the ripening ovary of small females and there is an observable difference in the white genital papilla which is more prominent in females.

There is a very noticeable difference in the maturation of fish reared in a common population and those reared in isolation. Fish reared separately grow uniformly and mature within a week or two of one another. Fish in populations grow unevenly and go through long periods where large individuals mature and others seem to be retarded. Only if the dominant fish are removed do others seem to mature and there are always "runts" which sometimes defy classification even after they are a year old.

These were never found in isolated young.

When the fry were sexed, males were moved into larger jars and females were put into tanks. All females from a spawn were put together unless they were to be held out as breeders. Adult fish which were not to be used further were disposed of by sale through a local tropical fish wholesale outlet.

Recording and identification

While various methods of tagging and marking large fish have been devised, aquarium fish are small and delicate so that most of these techniques are not suitable. It was necessary to devise a system to identify individual fish so that appropriate records of mating could be made. Individual fish were kept jarred rather than in populations except for certain special cases, as has been previously mentioned. Therefore jars were marked rather than the fish. Each jar carried a spawn source and an individual number.

All spawns were numbered serially and recorded in a journal with appropriate information concerning the parents, mating date, spawning date, locations and any other conditions of note. Spawning tanks were marked with basic data on adhesive labels.

A 3" by 5" record card was provided for each mating. This card was placed with the tank and then was moved with sets of jarred fry to an area of shelving restricted to the spawn. Some of the jars were marked as was the shelf in case the card might be accidently moved. Classifications were made and recorded on the cards. This information was then

transcribed onto a record form in a notebook. This form contained all information from the master journal plus the record card, and was left blank on one side where various calculations and comments could be added. The cards were filed for possible later reference.

The data recorded varied somewhat depending upon the objective. Early matings were made primarily to master techniques of breeding and rearing and to test genetic information obtained from the literature. After preliminary experience had been gained with the mutants previously described, fish were not always classified for these traits.

A substantial photographic record (mostly in color transparencies) was made of the many phenotypes extant. Color prints were obtained for use in this report. All processing was handled commercially by Eastman Kodak. Most of the photographs were taken with a Honeywell-Pentax Spotmatic 35mm camera using Kodachrome II color film. In order to get large enough images on 35mm film various close-up attachments were used. Fish were confined to a small tank especially designed to limit movement and the fish were allowed to see others in an adjoining container so that they would be stimulated to alertness. An electronic stroboscopic flash light source was used because bright steady lighting such as that provided by photo floods caused the fish to become frightened and to turn pale in color.

INVESTIGATIONS

Pigment cell studies:

Microscopic studies were made of the variations in order to determine more accurately what might be involved in the production of the gross phenotype. Pigment cells on scales were examined according to a modification of a pattern used by Wallbrunn (1958) where a "standard" scale was selected, located five rows ventral to the anterior edge of the dorsal fin. For illustrative purposes these scales were removed, placed in a drop of water on a slide, covered with a glass cover slip, and photographed in color. To keep the fish in a more or less normal physiological state while scales were removed they were anestheitized with Tricaine methanesulfonate (MS-222 Sandoz) and the scale was teased off with a fine dissecting needle. The fish did not appear to change visible color under this treatment and the scales came free with practically no effort. Most pigment cells on free scales were located in what has been called the "superficial zone" of the scale, that is, the portion of the scale that was not covered by other overlapping scales. While there were pigment cells located elsewhere, there were no types that did not also appear in the superficial zone. Therefore it was felt that this zone might be a reasonably representative sample. These cell populations are discussed in relation to the gross phenotype. To show details of color-producing elements, figures are provided with show standard scales at appropriate magnifications and details of cell types present.

Genetical methods

There has been no formal previous attempt to maintain stocks of any of the known mutant forms; thus it was not possible to obtain inbred or "pure" stocks. The only known mutant genotypes available were those that had been shown to be homozygous recessive. The new varieties studied were not described or even recognized at all well, so that stocks of these were also unavailable. It was necessary, therefore, to attempt to determine in what way a new phenotype might be different from the standard, then to make matings involving the new type with some individual which was normal with regard to the variation involved.

Given the standard normal phenotype it was possible to consider new types as modifications of this basic standard. Any one of the component colors might be enhanced, redistributed, reduced or entirely removed and, of course, there could be various combinations of these altered elements. For example, consider a "yellow" phenotype. A known mutant, "Cambodia", could cause the lack of black pigment. Some other mutants would have to be responsible for increase in yellow and the apparent absence of red. A test mating could be set up then of a yellow with any fish which had normal red and the investigation in question could then be limited to one element, red, or the lack of it.

A continuous search has been made for unusual types of fish and about a dozen and a half have been obtained over the period during which this study has been carried on. As a result, extensive information has been obtained concerning some variations while results are limited for others. Some of the information from more recent matings, which may have

seemed unrelated at first, have reinforced earlier findings from other lines of investigations. The exact system of matings for each individual element is therefore variable. Subsequent sections will deal with these elements and the matings related to them specifically.

Completion of genetical analysis logically leads to choice of gene symbols. In conformity with modern practice, the mutant will be designated with a mnemonic letter, which is capitalized if dominant or partially dominant to the standard, whose alleles are simply indicated by "+". The priority of previous symbolism is recognized and followed to the end of the presentation at which time all symbols will be reviewed and recommended changes suggested.

Sex ratio study

The matings involving the variables under consideration with reference to the sex ratio problem were handled in the manner previously described with certain modifications. From the experience obtained in early matings I felt that the following three areas had the greatest potential for further investigation: Relative parental age, low and high temperature, and "water" variation.

In order to provide a statistical system in which the investigation would be physically and mechanically feasible a routine was designed involving a grid of twelve individual spawnings. Mating activity is supposed to be optimum at 80°F (Goodrich, 1934) but it tapers off rather markedly within a few degrees above or below this. My contrasting experimental temperatures were to be 2°F or more above optimum and at least 2°F below

optimum. Six tanks were kept in a "warm" room where the temperature of the room was high enough to maintain tanks at 82°F even if tank heaters did not operate and the other six tanks in a "cool" room where room temperature was low enough that only tank heaters could get the water temperatures up to 78°F.

Within the two temperature levels it was then possible to set up a second variable. This I chose to call a "water" variable. Many factors can contribute to differences in water, e.g., its source, its treatment, and chemical reactions that may occur spontaneously with the presence of organisms such as fish and protozoa. In view of the fact that the test was going to require a considerable time span I felt it would be practically impossible to set up any specific controlled water condition. It seemed more logical to make a "screening" test to see if it might be shown that water had any effect of significance with the idea of making a more specific later investigation if anything proved significant.

Therefore water was used from two sources. These will subsequently be referred to as "Ames" and "Des Moines" water. The Ames source was not the city water but the Iowa State University tap water, from local deep wells. Sample analyses of these waters reveal comparative constituents as shown in Table 1.

Since there was variation from sample to sample, the only real element for comparison seemed to be "relative hardness". Ames water was then considered hard and Des Moines water soft. The experiments were performed near Ankeny, Iowa. Des Moines water was obtained from a tap and stored in glass or plastic containers. Ames water was carried to

Table 1. Comparative sample analyses of Ames and Des Moines water.

Characteristic	Ames 1961*	Ames 1967	DM 1964	DM 1967
pH	7.7	7.72	9.65**	7.48
Total hardness	418 ppm	380 ppm	92 ppm	86 ppm
Total alkalinity	286 "	278 "	49 "	36 "
Non-carbonate hardness as $CaCO_3$	132 "	102 "	43 "	50 "
Calcium (as calcium)	110 "	99.4 "	18.3 "	19.2 "
Magnesium (as magnesium)	36 "	32.1 "	11.5 "	9.2 "

* This analysis was provided by the Iowa State University Physical Plant Department. The other analyses were provided by the Des Moines Municipal Water Works Laboratory.

** The chemist at the Des Moines water works stated that the softening treatment of the Des Moines water produced high initial p^H readings but that they adjusted naturally in the distribution system. The second Des Moines reading was obtained from water which had been allowed to stand several days in a capped jar before analysis. Water used in experiments was always allowed to stand prior to introducing fish and periodic checks of pH always read in the 7-8 range.

the site in glass or plastic containers and stored in the same manner.

Spawnings were made so that eggs developed, hatched, and the fry were reared in each water type. New water of the appropriate type was used in jars initially and for changes. Fish were never transferred to other water until they had been sexed.

The variable of parental age seemed to be one of the most promising.

Three sets of matings were selected. These, too, were set up arbitrarily. The fish were considered "young" up until the time they were a year old and "old" after they were over a year old. Mating combinations were established as follows:

1. Young male X young female
2. Young male X old female
3. Old male X young female

While this does not include all possible combinations it was felt that it would provide suitable variations for a screening test. The interacting combinations of the variables selected required the setting up of twelve spawns for each "run". A "run" included each of the three age combinations in each of the two water types in each of two temperatures.

To observe repeatability of any obtained effect three replications were scheduled. In practice, it was not possible to obtain successful spawnings at every attempt. In addition, infection by a protozoan parasite (apparently Oodinium) which is very virulent in Bettas often killed spawns which were developing. It was necessary to repeat many of the matings several times. As a result, the possibility of comparing seasonal effects, for example, as a possible factor did not develop. Also no useful consideration of genetic effects is possible from these experiments, because of the necessity of selecting breeders within the proper age group. Both of these factors must certainly be suspected as additional possibilites for modification of the sex ratio.

While many spawns are very large the number of fry raised to maturity

was often less than 100 individuals. An arbitrary minimum of viable fry to be considered for the sex determination experiments was 50. The fry were jarred when approximately two weeks old so that there was no time for the development of cannibalistic behavior due to size or competitive differences. At that time they were about 5mm in length.

Because of differences in aggression and feeding, males often grow faster and females may be stunted. I assumed "average" sized fish would best represent a sample the unknown sex ratio of the spawn. The " middle" size range was sampled after stirring the fish around in the tank to break up possible congregations of like-sexed fish. Slightly larger fish were counterbalanced with slightly smaller. Extreme sizes were avoided. The sampling was subjective but size ranges at sampling time were not great.

Once the spawn had survived in good condition to the sampling stage it was considered a "good" run. While there was some mortality in jars in almost every spawn, and considerable mortality in a few, there was no reason to suspect differential mortality and the runs were considered satisfactory. In cases where samples fell short of 50 individuals they were considered for other genetic information they might yield but replaced in this series of experiments by a new spawn.

The fish were kept in these jars on an isolated section of shelving until they had reached classifiable maturity. Growth of the fins, color intensity and behavioral traits were considered sufficient to identify males. Females were not classified until egg-filled ovaries were visible through the side of the fish and the genital papilla was evident. While growth of fry populations is very variable, those in individual containers

grew at quite uniform rates and all reached sexual maturity at approximately the same time. On a few occasions there were individuals that did not mature rapidly. When it was not possible to positively identify these few (perhaps one in 200) they were tentatively classified as females because of their superficial appearance. It is not known why the maturation of these few was retarded but they developed into apparently normal fish eventually. For purposes of the experiment, the assumption was made that the normal sex ratio would be 1:1 and Chi-square tests were employed as the most suitable statistical tool. An analysis of variance was also made for the combined data.

RESULTS AND DISCUSSION

The consideration of color phenotypes is facilitated by a knowledge of the various kinds of pigment cells which may be present and their density, distribution and physiological state. I have presented background information suggesting that the pigments in Bettas appear to be melanin, melanin, erythropterin, lutein and guanine, and that the pigment cells appear to be melanophores, erythrophores, xanthophores, and guanophores (iridocytes).

Examination of scales of various phenotypes under dissecting and compound microscopes reveals considerable variety in the cell populations. The superficial zone is covered with a surface layer, which gradually slides off the scale under pressure of a cover slip, and the pigment cells, which appear to be firmly attached to the scale. (Figures 7, 8, and 9). The pigmented area may be totally devoid of pigment cells or range to dense coverage, depending on the fish. Distinct cell outlines are discernible for melanophores but red and yellow chromatophores are not as clearly defined, and guanophores, if they do in fact exist, are very poorly outlined.

Guanine crystals were often observed moving freely over the surface of the fish or moving off the scales. It is not possible to say they were not released by damage to cells but they were definitely free of any cell. Other pigment was never observed free of chromatophores. The guanine crystals appeared to be transparent and colorless unless light struck them at special angles. The light reflected was often bright, as light reflected from a mirror but at times it was almost prismatic. Green, blue,

Figure 7. Scales from various Betta color phenotypes at relatively low magnifications

a. Typical scale from the side of a Betta showing the pigmented superficial zone with melanophores in the contracted pigment phase. The posterior edge is uppermost in the figure, and has "comb teeth" at the margin. The "superficial zone" includes about a third of the "posterior field" and part of the middle but not including the "focus". (About 50X)

b. Scale from a dark fish showing melanophores with pigment in the spread or extended state which creates the darkest color. A few cells have their pigment contracted to the center of the cell. A general lightening occurs when all are contracted. (About 65X)

c. Scale from a yellow fish. Yellow fish have the Cambodia mutation and lack most melanophores. This scale has none. Yellow cells are present but do not show well in the reproduction. (100X)

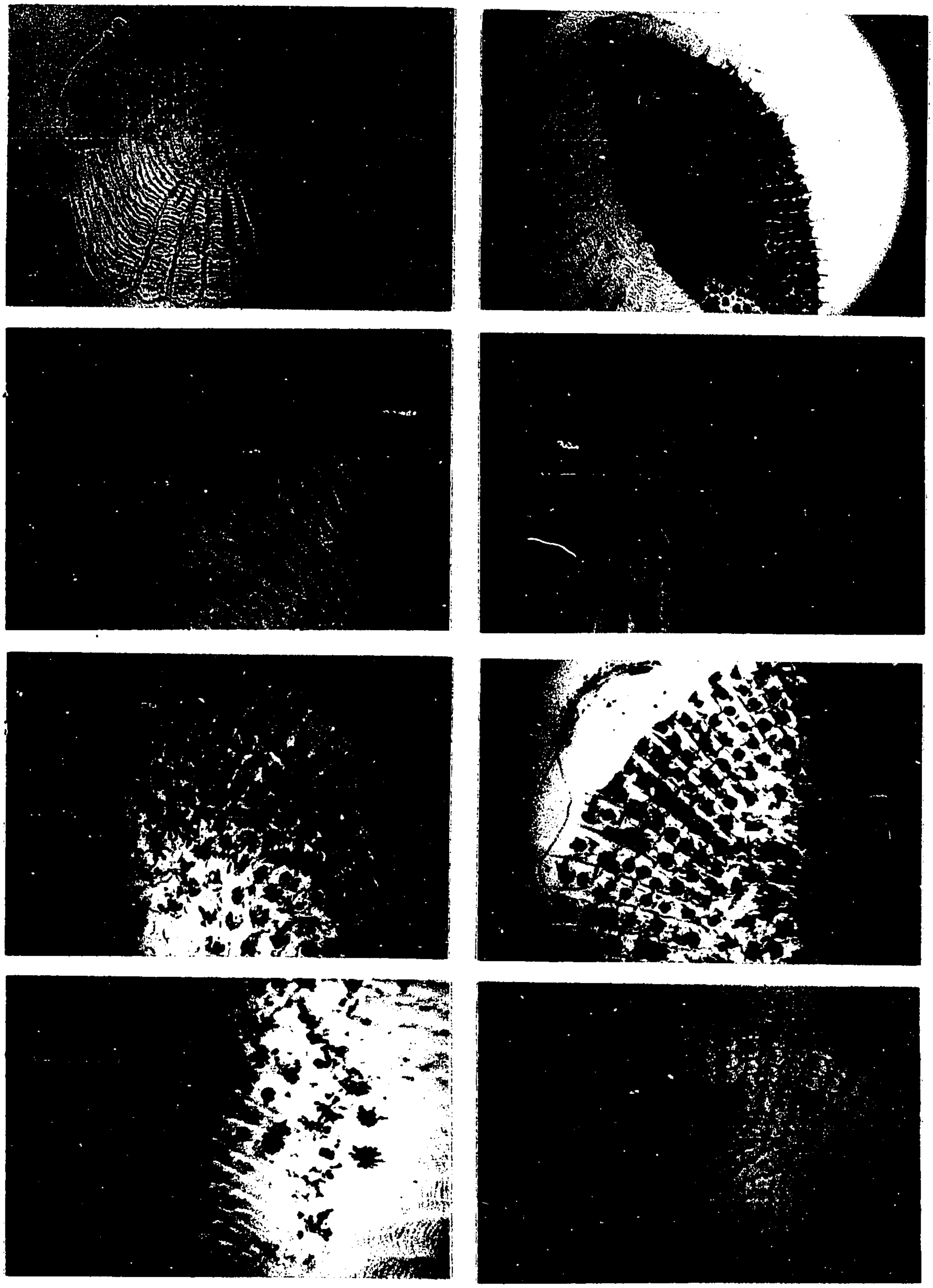
d. Scale from a red fish. Both melanophores and red cells are visible. (100X)

e. Scale from a "brownish" fish. Melanophores are visible in punctate, intermediate, and spread states. Reddish cells are barely visible. The material beyond the upper right surface is sliding from the scale surface (see text). (100X)

f. Scale from a blue fish. Only the melanophores and some red cells are visible. No guanine crystals show in the photographs at this magnification though the fish had high sheen. (100X)

g. Scale from a "black-yellow" fish. These fish show no red in the phenotype and cells are clearly melanophores and xanthophores. (100X)

h. Scale from a fish which lost its color. There are no visible pigment cells of any type. (100X)



a b c d e f g h

Figure 8. Scales from fish of various phenotypes at higher magnifications showing more cell detail.

a. Melanophores in the spread condition and practically completely hiding yellow cells. Wild type cell population. (440X)

b. Melanophores in the contracted state revealing yellow cells more clearly. Wild type cell population. (440X)

c. Melanophores and dendritic red cells. Some melanophores appear to have red color in their processes, others do not. Red cells seem to have replaced yellow. (440X)

d. Contracted melanophores and more clearly visible distinct red cells. Again, red cells seem to have replaced yellow. (440X)

e. Absence of melanophores and presence of xanthophores in scale from a yellow phenotype. (440X)

f. Spread and contracted melanophores and xanthophores from a "black-yellow" fish. (440X)

g. Contracted melanophores and xanthophores almost obscured by large numbers of guanine crystals. Note random distribution of guanine crystals. (440X)

h. Posterior field area of scale from fish which has lost its color. No pigment cells are visible. (440X)


a


b


c


d


e


f


g


h

Figure 9. Pigment cells from preparations viewed under oil immersion. Cells on scales from various phenotypes.

a. Melanophores in extended state. Nuclei and melanosomes are clearly visible as are "psoudopodia". (970X)

b. Cells from a red fish. Note reddish appearance of dendritic cells, and apparent dark central areas. (970X)

c. Distinct black and yellow cells as might be found in wild type or "black-yellow" phenotypes. (970X)

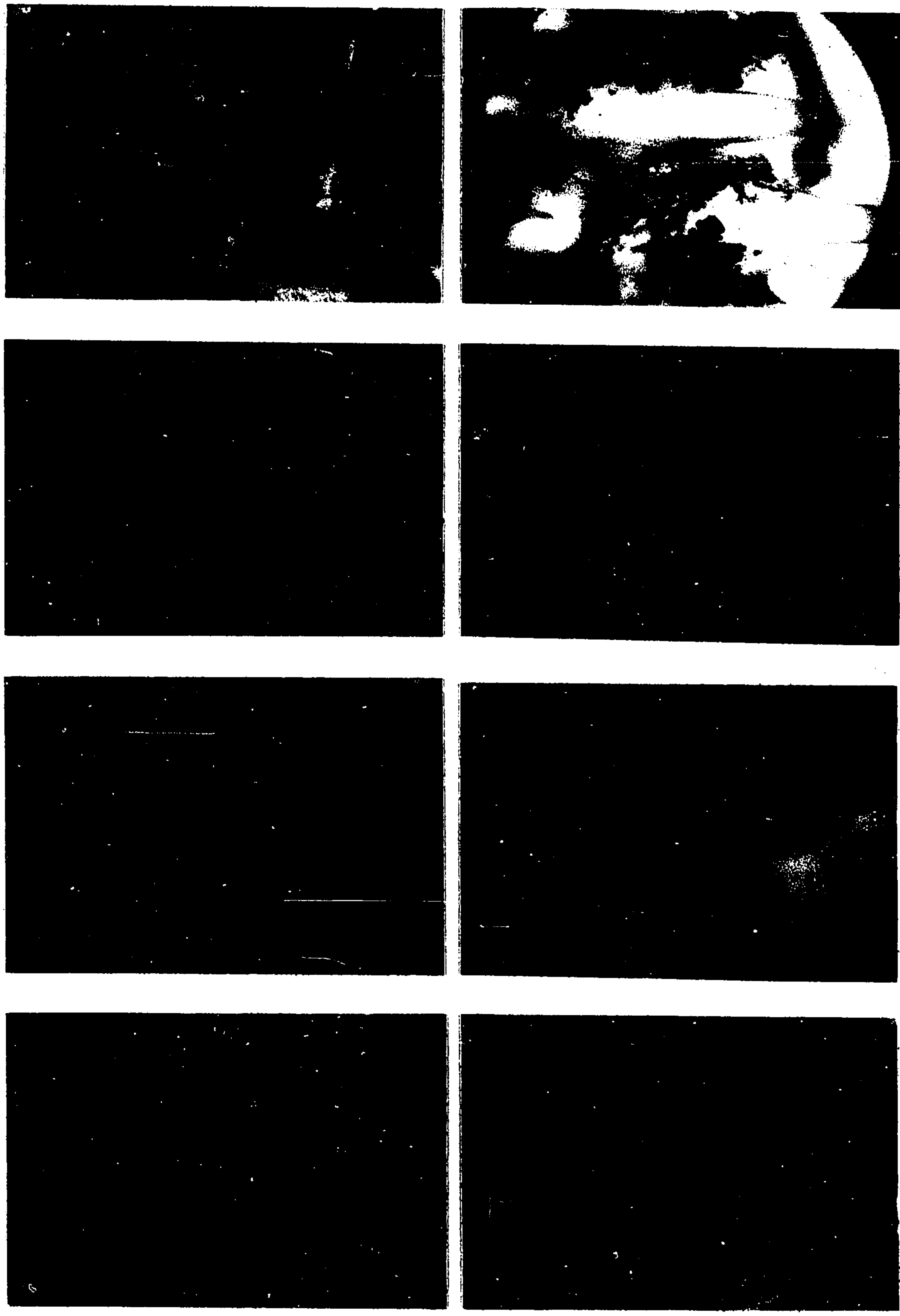
d. Distinct black and red cells as observed in scales from some red fish. (970X)

e. Yellow cells from a yellow fish. Denser central areas appear slightly orange to reddish. (970X)

f. Yellowish-red cells in a dark fish. While the cells do not show clearly in the print, the reddish areas appear to be reddish granules in yellow cells. (970X)

g. Guanine crystals from a fish with high iridescent color. Note the rather random distribution of the crystals. (970X)

h. Field from a fish which lost its color. Note absence of pigment elements. At right of field is what appears to be a cell nucleus, possibly from an epithelial cell. (970X)



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pink, yellow, rose, and lavender effects were visible, though the phenotypes attributed to them are rather uniformly green and blue. While Goodrich (1934) described them as "hexagonal crystals" I found them to lack uniformity of shape or size. They might roughly be described as hexagonal but generally one side is shorter and they lack well-defined corners. They appear to be thin plaques or plates which, under specific lighting look wavy or rippled with alternating light and dark bands.

Though the cells are called iridocytes and I refer to the color effect as "iridocyte color" there is some question in my mind whether it is proper to refer to it as such. Iridescence is a structural effect which causes changing color. The color of blue fish never changes and green only change slightly, sometimes appearing blue-green if viewed from certain angles. I am of the opinion that the blues may be the result of a Tyndall blue, viewed against a dark screen of melanophores. The presence of yellow might logically cause the blue to appear green. When a sugar-formaldehyde preserving solution was dripped on a green fish it immediately turned blue, an effect which might easily have been produced if the hypertonicity of the sugar solution were to somehow reduce the influence of the yellow. Whether this change was in the pigment cells, in the surface slime layer, or perhaps in the underlying tissue is not yet known.

The colors produced by pigments are also puzzling. The scales on the body of wild-type fish show only yellow and black cells. Examination of scales from reddish and brownish fish does not reveal additional cell types along with the normal. It does reveal apparent alteration in the

normal cells or the absence of one or more colors though this may be due to either absence of the cells or inability of the cells to produce pigment.

An investigation is required of the pigment units of the cells. Goodrich's report (1941) that red is erythropterin does not mention which phenotypes were analysed. Scrutiny of the cells in the various phenotypes reveals granular bodies. These are logically termed melanosomes in the black pigment cells but what they might be called in others is not clear. Granular objects which had no color were observed in yellow cells. Some yellow cells had granules that were red. Red cells were observed which contained red granules while other red cells had dark, almost black granules. Melanophores contained granules that were very black but at other times were not so dark, tending to appear brownish or yellowish and more transparent. All of the granules appeared to be similar in shape and size.

One might speculate that there are only two cell types which actually produce pigment, melanophores and xanthophores. Further, it could very well be that each of these may be variable and able to contain a reddish pigment under specific conditions. Brumbaugh (personal communication, 1968) postulates different kinds of melanin in association with melanosomes (granules) which develop at different rates in fowl, and relates this to chemical structure. It is tempting to consider the same possibility for melanosomes of Bettas.

Yellow cells, as described by Goodrich (1941) sometimes do contain red pigment. In Xiphophorus helleri he found yellow xanthophores in some color types but cells he called "xantho-erythrophores" in others.

He stated (p. 574):

> .....in the red form the yellow and red pigments are in the same type of cell which is called a xantho-erythrophore....., the presence of the red producing gene brings about the added development of the red pigment in cells which we consider to be homologous to the xanthophore of other types..... The xantho-erythrophores are characterized by dense yellow pigment at the center and a red pigment at the periphery of the main body of the cell, but yellow pigment may be diffused in the cytoplasm..... The red pigment is in the form of granules which may collect on the surface of the droplets of yellow pigment or be free in the cytoplasm.

Later, Wallbrunn (1958, p. 289) concluded that in the Betta:

> There are no chromatophores containing two pigments such as the xanthoerythrophores of *Xiphophorus helleri*.

Wallbrunn's statement is associated with his consideration of Goodrich's report and it is questionable that it was Wallbrunn's personal observation. I would guess that Goodrich observed only a few phenotypes. Figure 9-c shows clearly yellow and black cells but Figure 9-e shows yellow cells which look at least slightly reddish due to included granules and Figure 9-d and 9-f show red cells which are either new cells replacing the yellow or yellow cells filled with red pigment. Cells which might properly be called xantho-erythrophores do occur in some Betta phenotypes though Goodrich may not have seen them. Figure 10 shows the various types of pigment cells, in diagrammatic form, which I believe to exist in Bettas.

An idea of the overall effects of various combinations of chromatophores in various conditions may be obtained from Figures 11, 12, and 13. With four basic color elements and their potential absence or extension

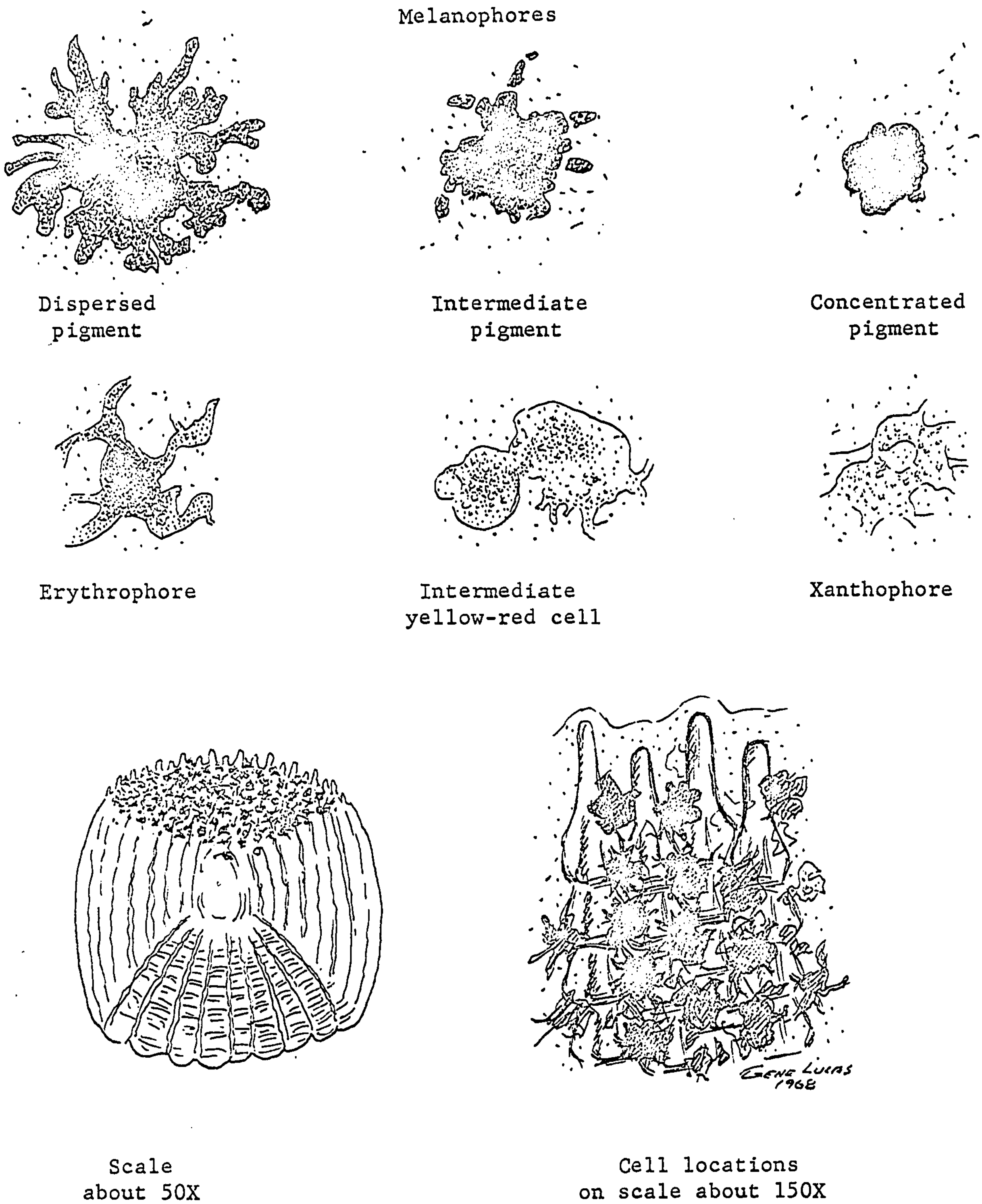


Figure 10. Betta pigment cells

Figure 11. Areas from assorted Betta phenotypes illustrating color variations as produced by different amounts of the basic colors

a. Region of the head of a Cambodia fish with red on the body and head and lack of melanophores. (About 35X)

b. Female with extension of green iridocyte color showing how color is patterned to scales. (About 1.5X)

c. A blond Cambodia showing especially well the many pigment cell types and silvery areas produced by guanine crystals localized to conform to the scale pattern. (About 30X)

d. Greater magnification of the same fish as in b. The greenish-yellow color is more evident as is the particulate nature of iridocyte color which appears as masses of tiny specks on the scales. (About 10X)

e. A Cambodia fish, lacking melanophores but possessing numerous red cells and large amounts of steel blue iridocyte color. The field includes the juncture of the anal fin and body. (About 20X)

f. A red, blond Cambodia. Some patches of melanophores are visible along with individual black and red cells. Iridocyte color is confined to very limited areas on the body and limited streaks between the fin rays. (About 20X)

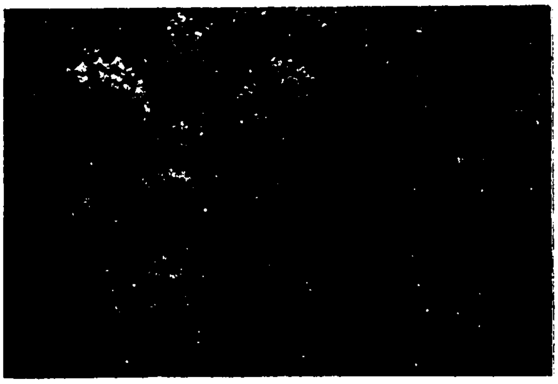
g. A highly pigmented dorsal fin viewed with transmitted light. The cells are dense enough to block light completely in a considerable portion of the fin. (About 15X)

h. The same fin area viewed with reflected light. The relative density of guanine is clearly defined as dark areas correspond to light areas in the previous picture. (About 15X)

a



b

c



d

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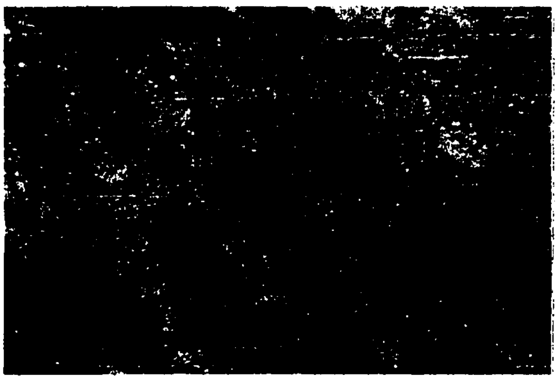

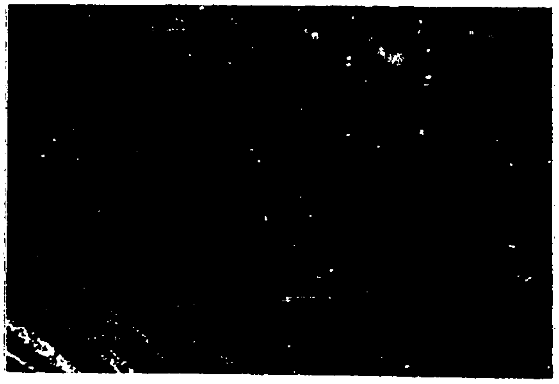
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Figure 12. Cell populations on caudal fins from various Betta phenotypes

a. Caudal fin from a red fish. Dense populations of red and black cells are present and almost occluding the transmitted light. (About 35X)

b. Caudal fin of a nearly colorless fish. The proximal portion of the fin was yellow. Only a few isolated pigmented cells are visible. (About 35X)

c. Caudal fin of a brownish-black fish with all melanophores having their pigment contracted. Large numbers of cells are clearly visible. (About 35X)

d. Caudal fin of another light colored fish. This one has more melanophores than the fish in b but they are still sparse.

e. Caudal fin area of the same brownish-black fish shown in c. Some red cells are present but the only ones in significant numbers are the punctate melanophores. Detail of the fin structure is shown by the articulated soft rays which have oval scales associated with them. There are no scales on web-skin between the rays. (About 100X)

f. A similar portion of the caudal fin of a reddish-brown fish. Both red and black cells are visible in the contracted state. Some red cells in the extended state may be seen in the center of the web. (About 90X)

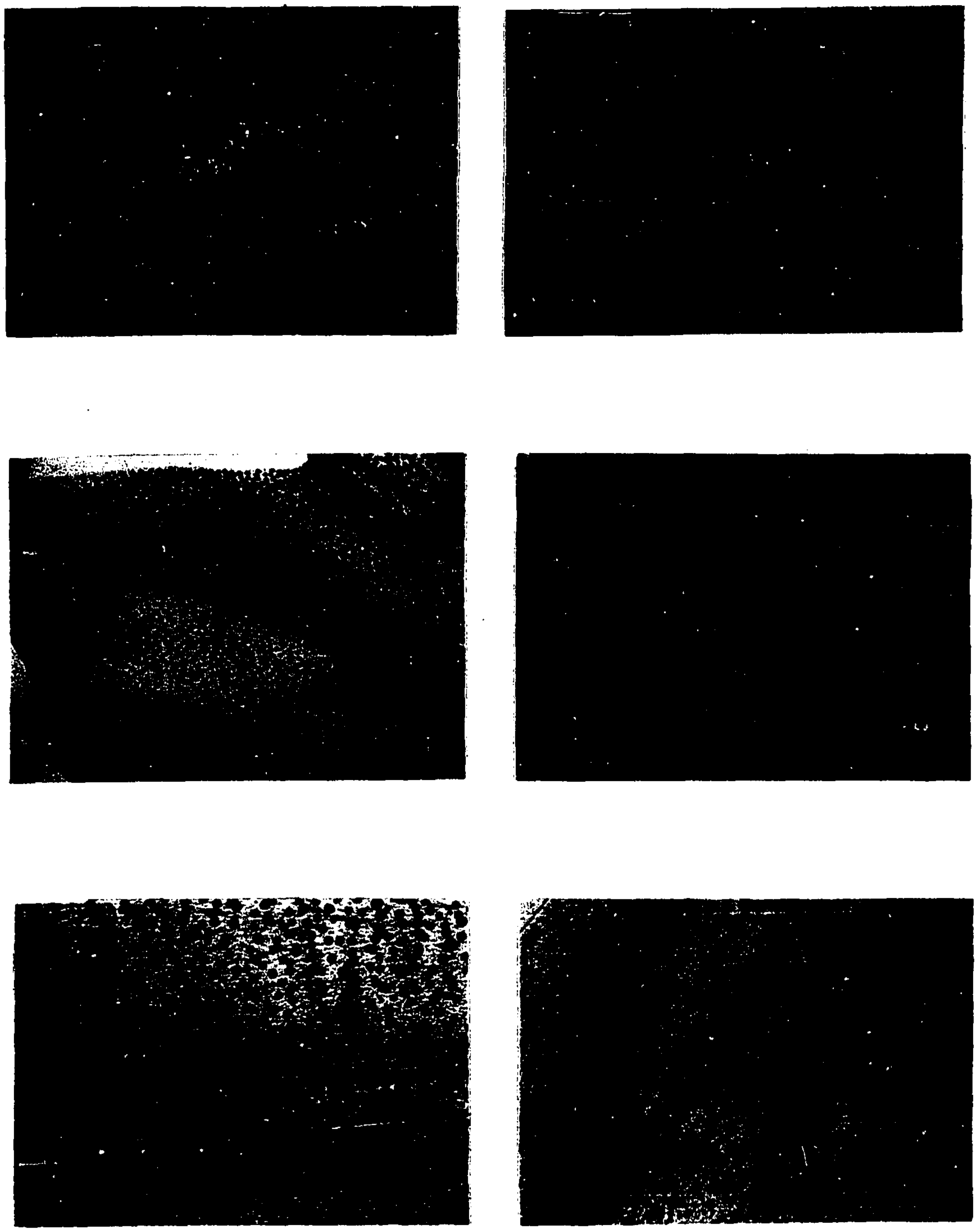

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Figure 13. Unusual physiological states of pigment cells in areas of Bettas

a. Head of a red, blond Cambodia following injection with epinephrin. Pigment cells rapidly contracted and the fish blanched to a very pale color. Punctate pigment cells are visible. (About 8X)

b. The body at the juncture of the anal fin. The blanched area is well defined from the area with normal pigment cells. (About 8X)

c. Another view of the line of demarcation between contracted cells and normal. Pigment cells are visible in the blanched area. Patterns of cell groups, both black and red, are visible. The fish has very limited iridocyte color. (About 8X)

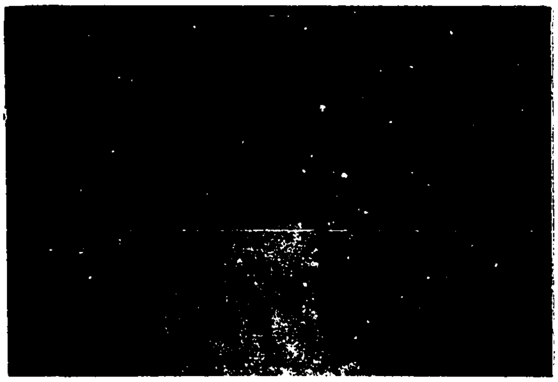
d. Enlarged portion of the normal area of the same fish. Melanophores of this phenotype are primarily bunched over the posterior field of the scales. The dark area on the left shows the "teeth" of the posterior margin of the ctenoid scale. Distinct individual red cells are visible in other areas, as are a few granules of guanine. (About 35X)

e. Caudal fin of a brownish-black fish showing a patch of red which appeared in the mature fish. The red cells in this region appear normal. Note branched, articulated soft rays of fin. (About 10X)

f. Caudal fin of another brownish-black fish showing a patch of yellow which appeared in the mature fish. This area contains what appear to be yellow cells but black cells have degenerated and are no longer present. (About 75X)

a

b



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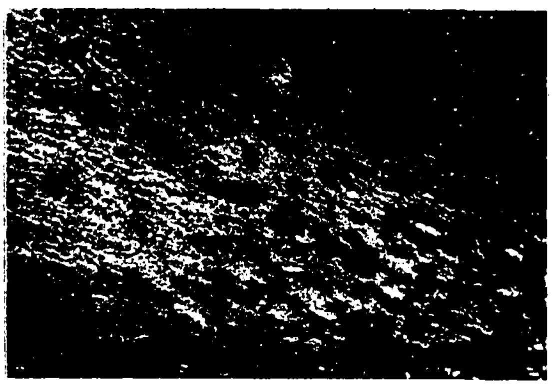

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and reduction (with variations) if present, a considerable number of interaction phenotypes is possible. Actually, not all of these possibilities have been discovered. The problem of color analysis remains complex, and the establishment of a standard of reference was necessary.

Wild Type *Betta splendens*

The standard selected and the wild fish obtained were quite similar in color but somewhat different morphologically. The wild fish have short fins and appear slightly thin but this may be due to nutritional rather than genetic differences. Genetic differences could be reasonably expected since domestic stocks have probably been isolated for forty years or more. Identification of wild stocks as *Betta splendens* was based upon color, morphology and reproductive behavior. Crosses of wild with domestic stocks were successfully achieved.

Scale and fin ray counts for both forms are within the described ranges. Body lengths of male wild fish averaged 4-6 cm though they may grow larger. Domestic males reach 6-7 cm. The striking difference occurs in the finnage. The caudal fins of wild fish are uniformly about 1-1.5 cm while domestic forms have caudals which are routinely 4-5 cm and on fine specimens may reach 8-9 cm. The dorsal, anal, and pelvic fins are similarly elongated in domestic fish.

The pigment distribution on wild fish must be known for accurate comparison with domestic color abnormalities. Melanophores are distributed over the entire surface. Larger amounts of black are found along the margins of scales and in aggregations approximately covering the posterior

fields of the scales, along the rays of the fins, at the distal margins of the medial fins, on the proximal portion of the pelvic (ventral) fins, and in bands in the inter-ray webbing of the dorsal Black is almost totally absent from the inter-ray webbing of the anal and caudal fins, the pectorals, the distal portion of the pelvics, and an area along the posterior margin of the operculum.

Red pigment cells are confined to the proximal two-thirds of the pelvic fins, the distal third of the caudal, the distal portion of the posterior one-third (approximately) of the anal, and the superior half of the light area mentioned above on the operculum. No red cells were observed on body scales. Yellow pigment cells are found throughout on the body scales and on visible areas of the colored fins (the pectoral fins normally are unpigmented) though they may be hidden by darker or more opaque cells.

The iridocyte color is green in all specimens I obtained from Vietnam. The fish received earlier from Germany possessed iridocyte color variations found in domestic stocks. The previous descriptions fail to mention color polymorphism in wild fish and observation of the Vietnamese fish show none; therefore I suspect that the German fish had been crossed with mutant stocks at some time or other. Perhaps they had escaped or been released as poor grade because of their short finnage. Iridocyte color lies most superficial and masks all other color elements. In the wild fish it is limited to specific areas. The anterior central area of the exposed surfaces of the scales contains a spot. These spots are very evident throughout the length of the regular scale row just dorsal to the lateral line, not quite so evident in the next superior row, and hardly

noticeable in the others except for some in the posterior quarter of the body. A web-like pattern is well formed on the dorsal fin, as are radiating bands on the inter-ray webbing of the caudal and anal fins. There are spots on the posterior and inferior portions of the iris of the eye.

The general effect in a quiescent fish is rather drab as none of the colors appear intense. In display, however, a rather remarkable transformation takes place. The melanophore pigmentation spreads and the fish becomes very dark. Black patterns disappear. At the same time the red appears darker and more intense and the iridocyte color becomes brilliant by contrast. Dr. George Myers (1947) described the male as follows:

> He is a dark seal-brown to blackish in color, and the tiny metallic yellow pin-points on his sides show out like diamond crumbs on black velvet. His spread, fan-like dorsal fin is brilliant metallic yellow, covered with black flecks. His perfectly round, spread tail is deep crimson edged with black, and along the fanned out rays are wide, intense prismatic, canary yellow streaks, turning to fiery blue as the fish turns and the light strikes them in a different direction. The wide anal fin is basally red, turning to dull yellow towards the rear and edge, and each ray is narrowly picked out in black. His long ventral fins are deep crimson, tipped black, and this again tipped on the very end with yellow white. His gill membranes, which are constantly protruded and retracted as he wheels about and displays are deep crimson and black and on his gill covers there is a metallic spot. Finally, his black head is set off by a fiery, metallic green eye.

The photographs in Figure 1 (p. 11) are of a single fish in three stages of coloration. With light coming from in front and above, the iridocyte color is yellowish green on the dorsal, shades to a true green on the body and finally to a blue-green on the anal and caudal fins. The pictures depict quite well the actual coloring, which, in the 18 males I have examined, is quite uniform. None really look yellow though the green sometimes is more yellow in the dorsal part of the fish. Myers'

description was for a popular magazine so may have been exaggerated to a degree but there is no question that the fish becomes a scintillating beauty. The transient striping and banding patterns on Bettas are under neural or hormonal control. Marrone and others (1966) found that norepinephrin would evoke intensification of color while I have observed that epinephrin caused blanching (see Figures 13-a, 13-b, 13-c, p. 91). It may be that there is a greater influence due to neural control than might be expected, or the principal color expanding agent may be one of the pituitary hormones, MSH or intermedin.

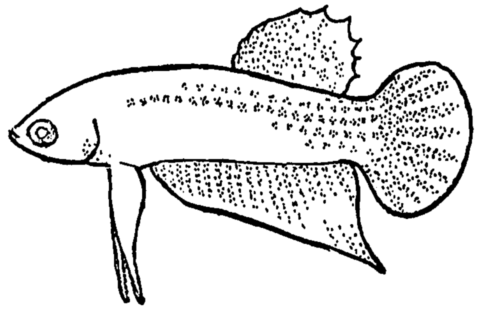
The basic tissue substance contributes to the visual phenotype. It is similar in Bettas to that of other small fish. Muscle tissue is partially transparent, grayish yellow. Internal parts may look yellowish white if guanine deposits are extensive or they may be slightly pinkish due to blood.

It seemed necessary to separate component elements of the wild type phenotype for comparison with the various mutants; the separation is shown diagrammatically in Figure 14. It was then possible to consider variations of specific components with normal rather than complex phenotypes resulting from various interactions.

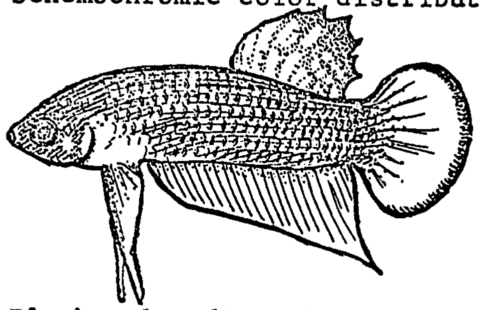
Investigations of Deviant Forms

Schemochromic colors

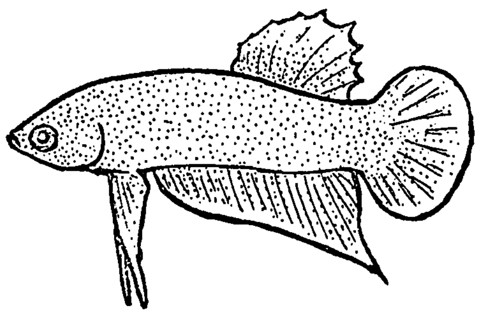
It was necessary to establish two kinds of normality for the schemochromic colors of Bettas; 1) the color or hue itself, and 2) the density



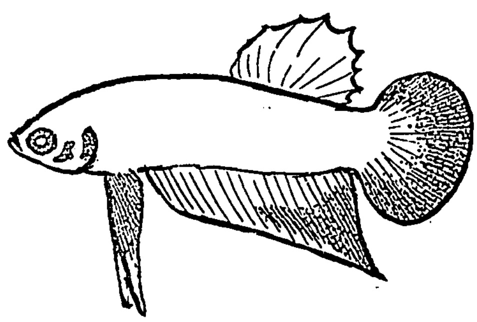
a. Schemochromic color distribution



b. Black color distribution



c. Yellow color distribution



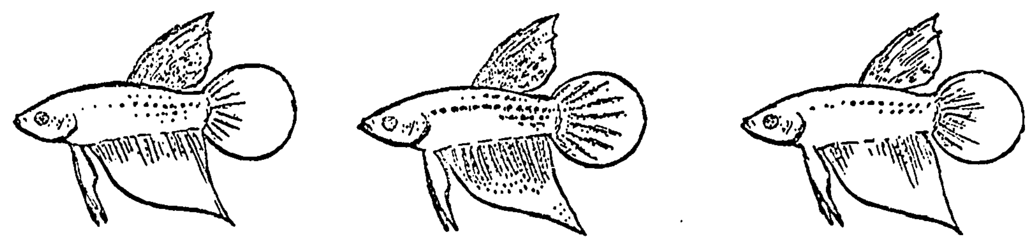
d. Red color distribution

Figure 14. Distribution of the four principal color forming elements of normal or standard <u>Betta splendens</u>

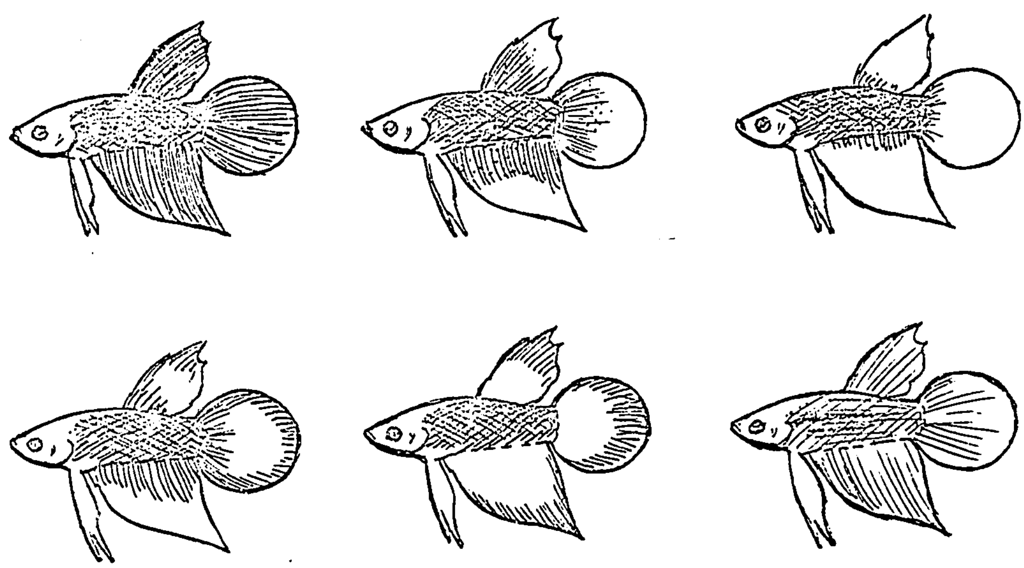
and distribution. Consideration of literature descriptions and observations of living specimens indicate that the normal hue is green rather than blue and the normal distribution is limited to the streaks and spots described here for the wild type. The deviant forms are the two shades of blue and the highly metallic sheen due to extensive spread of iridocyte color. Classification of the greens vs. blues is not difficult in most specimens and quite easy in the ones having extensive iridocyte color. Classifications for the spread is more difficult, as the trait is extremely variable both in area and density. Some samples of possible variations are shown in Figure 15. With practice one can classify the fish into two broad groups, which have been termed "iridescent" and "non-iridescent", but which I feel should more correctly be referred to as "spread iridocyte color" and "normal iridocyte color". These variations have already been described and are shown in Figure 6 (p. 40).

<u>The blues and greens</u> Although previous studies seem to have firmly established the proper pattern of inheritance of these colors many spawns were routinely classified for them. Four matings of green X green individuals produced only green color in 137 reared progeny. No matings of steel blue X steel blue were made but a number of other combinations were, including steel blue X green (and reciprocals), and blue X green (and reciprocals), blue X steel blue (and reciprocals), and blue X blue. These matings include stocks from many sources in various combinations. Where possible, specific information is given in the tables. Results of matings between steel blue X green parents appear in Table 2.

A total of seventeen spawns were classified from blue X green matings (Table 3).



a. Diagrams of fish with variations in the normal limited spread of iridocyte color.



b. Fish with variations of the extended spread of iridocyte color. Note that the head region and the pelvic (ventral) fins rarely possess this color.

Figure 15. Variations in the distribution of iridocyte color in the two main classification groups (shaded areas indicate only iridocyte color)

Table 2. Spawns involving one green and one steel blue parent

Spawn Number	Parents Male	Parents Female	Progeny Steel blue	Progeny Blue	Progeny Green
	Green	Steel blue			
78	"	"	0	333	0
	Steel blue	Green			
118	"	"	0	19	0
119	"	"	0	59	0
147	"	"	0	53	0
Totals			0	464	0

Table 3. Spawns involving one green and one blue parent

Spawn Number	Parents Male	Parents Female	Progeny Steel blue	Progeny Blue	Progeny Green
	Blue	Green			
3	"	"	0	6	3
42	"	"	0	14	23
58	"	"	0	47	44
64	"	"	0	26	27
67	"	"	0	18	20
110	"	"	0	13	12
117	"	"	0	5	2
146	"	"	0	9	8
171	"	"	0	5	10
172	"	"	0	11	10
173	"	"	0	22	19
Sub-totals			0	176	178
	Green	Blue			
34	"	"	0	6	9
69	"	"	0	204	140
76	"	"	0	46	49
103	"	"	0	109	134
165	"	"	0	36	52
205	"	"	0	23	24
Sub-total			0	424	408
Totals			0	600	586

Expected ratio: 1:1 (593:593)
Observed: 600:586
Chi-square = .164 df = 1 P = 75 > 50%

Seven spawns were classified from blue X steel blue matings. (Table 4).

Table 4. Spawns involving one blue and one steel blue parent

Spawn Number	Parent Male	Female	Progeny		
	Blue	Steel blue	Steel blue	Blue	Green
45	"	"	284	292	0
	Steel blue	Blue			
72	"	"	113	93	0
73	"	"	150	135	0
84	"	"	5	3	0
120	"	"	5	6	0
121	"	"	8	11	0
175	"	"	7	3	0
Sub-total			288	251	0
Totals			572	543	0

Expected ratio: 1:1 (557.5:557.5)

Observed: 572:543

Chi-square = .754 df = 1 P = 50$>$25%

Both parents were blue in the remaining mating combination, the results of which appear in Table 5.

Table 5. Spawns in which both parents were blue. F_2 are separated from others which have individuals from various sources

Spawn Number	Parents Male	Parents Female	Progeny Steel blue	Progeny Blue	Progeny Green
	Blue	Blue			
(F_2 spawns)					
52	"	"	32	56	21
74	"	"	33	46	34
Sub-total			65	102	55
(Other sources)					
1	"	"	35	30	28
2	"	"	8	21	15
47	"	"	55	107	40
56	"	"	40	50	32
77	"	"	59	81	48
79	"	"	7	9	8
100A	"	"	5	18	10
193	"	"	6	7	5
199	"	"	10	25	8
202	"	"	15	13	8
208	"	"	17	23	10
209	"	"	6	17	18
210	"	"	3	7	12
Sub-total			266	408	242
Totals			331	510	297

Expected: 1:2:1 (284.5:569:284.5)
Observed: 331:510:297
Chi-square = 40.53 df = 2 $P < 1\%$ (Significant variation)

The results of these matings are consistent with the previous reports of Goodrich and Mercer (1934), Umrath (1939), Eberhardt (1941), and Wallbrunn (1958). All results reported here indicated that there are two alleles which are responsible for this color, and that there is incomplete dominance or codominance between the alleles. In all cases except the blue X blue matings the ratios were well within a satisfactory

statistical range as determined by the Chi-square test. I can see no clear explanation for the significant deviation in numbers obtained in the blue X blue matings (Table 5). The color classes are quite distinct and I have seen few fish from among probably 10,000 or more I have observed which I felt would have been difficult to classify for this trait. The color becomes visible when the fish are very small and many could be classified when they were only two or three weeks old. None were ever observed to change (iridocyte) color at any time other than to lose some of it.

If individual matings are considered much variation may be noted. Some spawns conform to the 1:2:1 ratio rather well while others do not. The F_2 matings are within the statistically acceptable range but the number is not very large. Lacking further information it may be reasonable to suggest that the variation is coincidental. Further data are necessary to clarify the problem.

While the mode of inheritance is not questioned, the designation of an allele which might, in the genetic sense, be considered a mutant, has never been attempted. Since the wild type fish and the designated standard are both green steel blue and blue should be considered abnormal. To modernize the symbolism I propose the symbol "Bl" indicating blue (of whichever type) for the co-dominant abnormal allele, instead of V employed by others, and + for the wild-type allele instead of v.

While additional further studies are necessary I would tentatively suggest that the mutant has some inhibitory affect on the development or expression of yellow color in the phenotype so what would normally

appear green now appears blue. The differences in the two blues are not properly explained and also require additional study.

<u>Spread iridocyte color</u> A number of spawns were classified for variation of these colors to help understand the somewhat confusing previous system of identification. The different phenotypes have not been considered as abnormal and normal conditions. Eberhardt's report (1941) discussed the variation within types and Wallbrunn (1948, 1951, 1958) attempted to clarify the descriptions. I have shown variations within the two principal types in Figure 15 (p. 98). I consider the low sheen form to be normal, since this is the condition of the wild fish, and the high sheen types as abnormal. The high sheen condition, being attributed to increased density and distribution of guanine, will be referred to as "Spread" iridocyte color as I consider the term most explicit.

In agreement with the conclusions of Eberhardt (1941) and of Wallbrunn (1951, 1958) I have found the "reduced iridocyte" or low-sheen (normal) condition to breed true, regardless of origin. Nine spawns were classified which had both parents normal (though in some cases spread was known in the ancestry). All 271 progeny reared were normal. Similarly the spread iridocyte class can breed true. Three spawns each had both parents and all grandparents of the spread type. All 694 progeny were spread, indicating that if this is a dominant condition at least one of the parents in each mating was homozygous. Additional matings were made involving normal X spread parents and spread X spread with both parents heterozygous. The results of these crosses appear in Tables 6, 7, and 8.

Table 6. Spawns from normal males mated with spread females classified as heterozygous spread because they had one normal parent

Spawn Number	Parents Male	Female	Progeny	
	Normal	Spread	Normal	Spread
72	"	"	64	57
77	"	"	90	98
84	"	"	5	4
146	"	"	8	9
Totals			186	168

Expected: 1:1 (167.5:167.5)
Observed: 167:168
The results cannot be closer

Table 7. Spawns from normal males X females classified as spread because of their phenotypes--they could not be classified homozygous or heterozygous from pedigree data

Spawn Number	Parents Male	Female	Progeny	
	Normal	Spread	Normal	Spread
(Female parent apparently homozygous)				
110A	"	"	0	49
147	"	"	0	53
(Female parent apparently heterozygous)				
117	"	"	3	4
118	" "	"	10	9
119	"	"	8	10
172	"	"	8	13
173	"	"	19	23
Totals of last five spawns			48	59

Expected: 1:1 (53.5:53.5)
Observed: 48:59
Chi-square = 1.130 df = 1 P = 50 $>$25%

Table 8. Spawns from parents classified heterozygous for spread, based on pedigree information and progeny types obtained

Spawn Number		Parents Male	Parents Female	Progeny	
		Spread	Spread	Normal	Spread
47		"	"	23	177
78		"	"	92	241
199		"	"	14	30
202B		"	"	7	29
207	(Siblings)	"	"	2	8
208		"	"	5	33
Totals				143	518

Expected: 3:1 (495.75:165.25)
Observed: 518:143
Chi-square = 4.0 df = 1 P = 5 > 2.5%

One mating of a spread male and a normal female produced a spawn containing 46 normal and 49 spread progeny, showing that the male was heterozygous.

The significant statistical deviation obtained in the spread X spread matings shown in Table 8, could be due to improper classification. However, it was no problem to classify most fish. There was a wide range of variation within classes, almost a continuum, as suggested by Wallbrunn (1958), but few fish fall into the intermediate range which may be improperly classified. There has been no evidence of differential viability and I feel that classification errors are quite rare. With the available data, the deviation can hardly be explained by any factor other than chance. The spread patterns conform adequately with the hypothesis of a single dominant mutant with variable expressivity. This variability has not been acceptably traced to specific modifiers though Wallbrunn

(1948) made one attempt.

Eberhardt's symbol ri refers to the normal type, and therefore is not appropriate as a symbol for a mutant. The spread of iridocyte color is abnormal, and thus, according to convention, should provide the symbol. I suggest the symbol Si, for spread iridocyte color, as a more logical substitute. This appropriately signifies the dominant and abnormal character of the variation.

Variations in Black Coloration

Matings were made to obtain information about three distinct variations in the development of black color. Two of these are the previously described Cambodia (p. 30 and Figure 4, p. 33) which almost completely inhibits the development of black color, and the "bright" or "blond" mutant described by Wallbrunn (p. 34 and Figure 5 p. 36) which reduces the amount of black but to a lesser degree. The third variation is a previously unreported extension of black which results in phenotypes much darker then normal.

Cambodia Many spawns were classified for this variation from a number of different mating combination. Six spawns from Cambodia X Cambodia parents of various origins yielded 1,085 progeny, all Cambodia type, showing that the abnormality breeds true. The results obtained from other mating combinations are presented in Tables 9, 10, 11, and 12.

The data clearly confirm all previous reports regarding the Cambodia mutant as a simple recessive. Though no mention was made of selection of

Table 9. Spawns from normal X normal matings in which one or both parents were homozygous for normal

Number of spawns	Parents: Male	Parents: Female	Progeny: Normal	Progeny: Cambodia
	Normal	Normal	Normal	Cambodia
29	Origin ?	Origin ?	2159	0
3	Origin ?	F_1*	29	0
1	Wild	Origin ?	40	0
2	Wild	Wild	53	0
1	Origin ?	Wild	10	0
Totals			2281	0

* from one Cambodia parent

Table 10. Spawns from Cambodia X normal matings giving only normal progeny

Spawn Number	Parents: Male	Parents: Female	Progeny: Normal	Progeny: Cambodia
	Cambodia	Normal	Normal	Cambodia
2	"	"	44	0
164	"	"	50	0
Sub-totals			94	0
	Normal	Cambodia		
72	"	"	206	0
197	"	"	13	0
Totals			313	0

Expected: All normal
Observed: All normal

Table 11. Spawns from Cambodia X normal matings giving some Cambodia progeny, or where the normal parent was a Cambodia cross

Spawn Number	Parents Male	Female	Progeny	
	Cambodia	Normal	Normal	Cambodia
1	"	"	48	57
3	"	"	9	0
56	"	"	64	58
64	"	"	28	25
100A	"	"	18	15
189	"	"	10	15
Sub-totals			177	170
	Normal	Cambodia		
58	"	"	52	39
Totals			229	209

Expected: 1:1 (219:219)

Observed: 229:209

Chi-square = .912 df = 1 P = 50>25%

Table 12. Spawns from normal X normal matings which produced some Cambodia progeny

Spawn Number	Parents Male	Female	Progeny Normal	Cambodia
	Normal	Normal	Normal	Cambodia
Sibling matings				
5	"	"	13	5
8	"	"	14	6
20	"	"	0	1
21	"	"	24	3
73	"	"	218	67
101	"	"	141	56
207	"	"	8	2
226	"	2	26	1
Sub-totals			444	141
Other matings				
30	"	"	17	2
42	"	"	26	11
47	"	"	152	50
448	"	"	5	1
66	"	"	27	16
76	"	"	73	22
79	"	"	13	11
119	"	"	12	6
172	"	"	13	8
210	"	"	21	1
224	"	"	20	6
225	"	"	1	4
243	"	"	37	12
Totals			861	291

Expected: 3:1 (864:288)
Observed: 861:291
Chi-square = .0416 df = 1 P = 90>75%

a symbol on the basis of abnormality the letter <u>c</u> (for Cambodia) designates the mutant form and is therefore appropriate and acceptable.

"Bright" or "blond Cambodia"   Only a few matings were classified for the mutation Wallbrunn called bright. Three spawns from bright X bright parents produced only bright progeny. From a normal (with no blond ancestry) X blond only normal progeny were obtained. Backcross and F_2 matings appear in Tables 13 and 14.

Table 13. Spawns from bright X normal (heterozygous)

Spawn Number	Parents Male	Parents Female	Progeny Normal	Progeny Bright
	Normal	Bright	Normal	Bright
47	"	"	81	71
61	"	"	80	66
64	"	"	7	21
Sub-totals			168	158
	Bright	Normal		
72	"	"	101	105
101	"	"	55	86
Totals			324	349
Expected:	1:1 (336.5:336.5)			
Observed:	324:349			
Chi-square	.92 df = 1	P = 50 25%		

Table 14. Spawn from normal (heterozygous) X normal (heterozygous) (F_2 of spawn #30 (Bright X normal))

Spawn Number	Parents Male	Parents Female	Progeny Normal	Progeny Bright
	Normal	Normal	Normal	Bright
52	"	"	71	38
Expected:	3:1 (81.75:27.25)			
Observed:	71:38			
Chi-square	5.65 df = 1	P = 2.5 1% (significant)		

The data support Wallbrunn's hypotheses of a mutant allele at a new locus, recessive to normal. The significant variation in the F_2 is attributed to chance as no other evidence is questionable and it is a single sample. His selection of the symbol b for bright designated the abnormality in indirect terms. It described the appearance of red, which looked different because of it, rather than suggesting the reduction of melanin. When Gordon interpreted Wallbrunn's symbol as "blond Cambodia" he related it to similar mutant in the guppy. Fortunately, since both bright and blond begin with b, both authors used the symbol b. Since there is no direct affect on red, bright is not suitable. There is no relation to Cambodia so blond Cambodia is also improper. Umrath (1939) used m (for reduced melanophores) which related to physiology but not well enough to the phenotype to become accepted. I suggest the retention of the b symbol and the adoption of "blond" as the best name and henceforth use this designation.

"Black" or melanistic Melanistic forms of *Betta splendens* have been found which have only been discussed briefly in popular literature. While these have been called black, the term melano seems more appropriate as melanistic fish are usually not very black. Various melanistic phenotypes are shown in Figures 16 and 17.

No genetic analyses have been published. Investigations were hindered somewhat at first because of the apparent sterility of all the melanistic females used. No verified reports of viable progeny from dark females have been found. They do spawn and some initial development of the embryo has been observed. I was unable to get any to hatch, though males appear to have normal fertility when mated to other females. The

Figure 16. Black or melanistic Bettas

a. A typical male. Iridocyte color is visible on the body. Black markings are still observable, especially on the dorsal fin. The "best" black color appears to be on portions of the fins, other areas being paler or brownish. (About .5X)

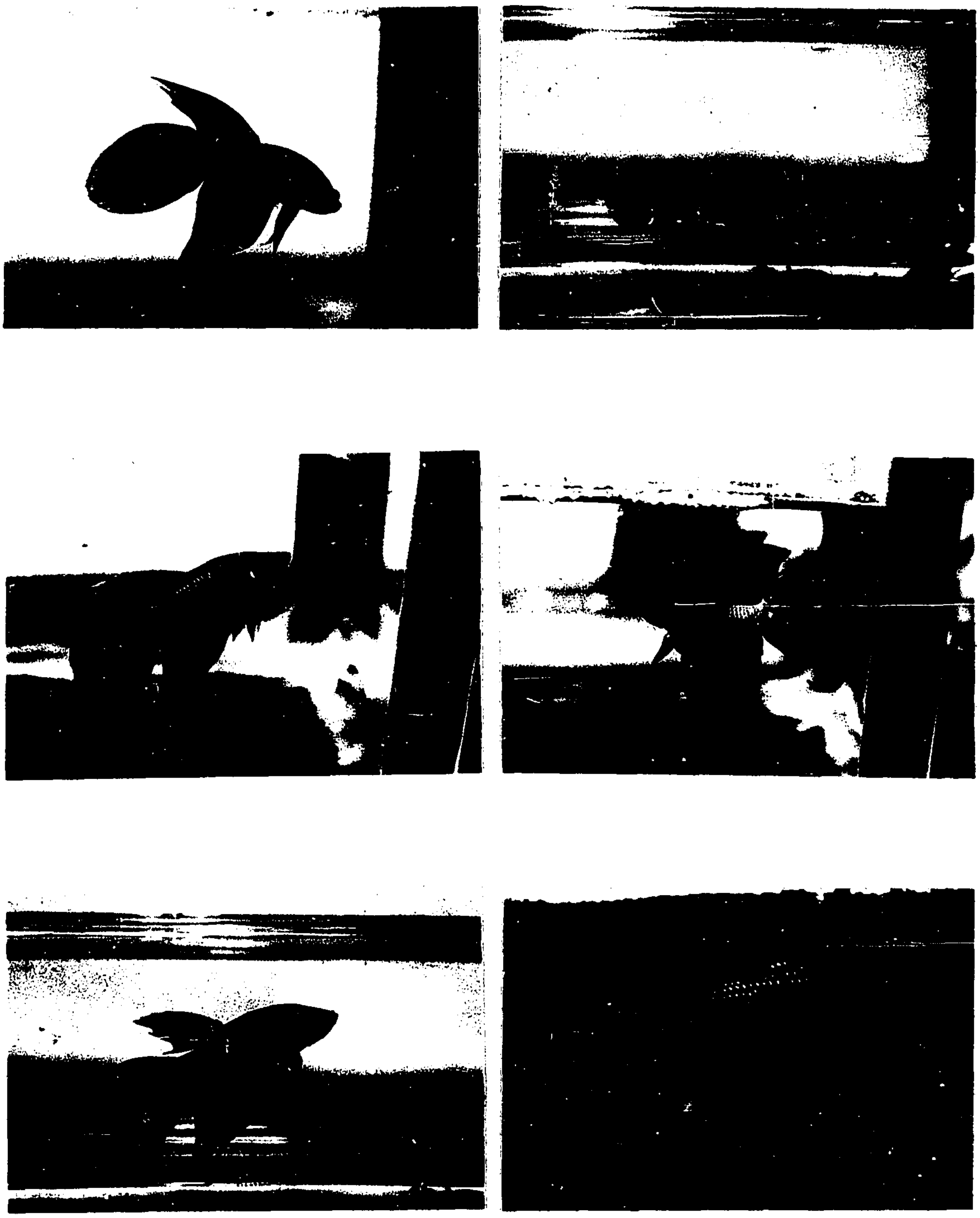
b. A typical female. All characteristic markings are visible though not as distinct as on normal fish. The color of the egg-filled ovary shows through the light transverse bands on the side. (Life-sized.)

c. A male with blue iridocyte color. Note brownish tinge. (About .5X)

d. A male with green iridocyte color. (About ½X)

e. A male with the normal non-spread condition for iridocyte color. (About .5X)

f. A male with normally distributed green iridocyte color photographed under reflected light. The brownish nature of the color is more visible. (About life sized.)



a b

c d

e f

Figure 17. Variations and unusual expressions of dark pigmentation

a. A male black with normal iridocyte color in "faded" condition which occurs when the fish are frightened or over light backgrounds. Longitudinal striping becomes visible. (About .5X)

b. A Cambodia female from a black father. She appears as any other Cambodia. (About 2X)

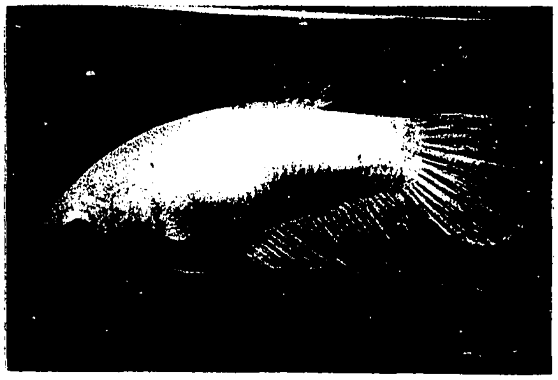
c. A brownish variation of the black type. (About .8X)

d. A black type male in a reddish state which develops under various conditions. Note "regular" black in background. (About .5X)

e. An unusual "pale" dark male. The fish is mildly melanistic. (About .8X)

f. The same fish, photographed against a light background. (About .8X)

a



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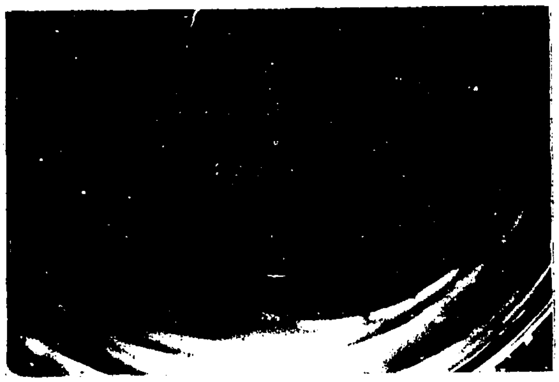

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melanistic types range through a series of light and dark brown and grays. However, the failure of eggs from all melanistic females to develop suggests that a single genetic type of melanism is responsible for all the many shades.

A number of spawns could be classified for possession or lack of melanism. Figure 18 is a pedigree chart of matings which led to or produced melanistic Bettas. Thirty-one matings of fish with normal ancestry produced no dark progeny; no melano X melano spawns were obtained. Table 15 reveals that melano males X normal females having no melano ancestry produce only normal progeny, showing that the abnormality is recessive.

Table 15. Matings between melanistic males and normal females having no melano ancestry

Spawn Number	Parents Male	Parents Female	Progeny Normal	Progeny Melano
	Melano	Normal	Normal	Melano
WM	"	"	ALL	0*
JH	"	"	ALL	0*
71	"	"	2	0
103	"	"	243	0
123	"	(Wild) "	10	0
197	"	"	13	0
Totals			268	0

Observed: All normal

* Information from other breeders.

Tables 16 and 17 contain the results of matings between F_1 males X females that were normal, the first having no known melanism in the ancestry and the second having it.

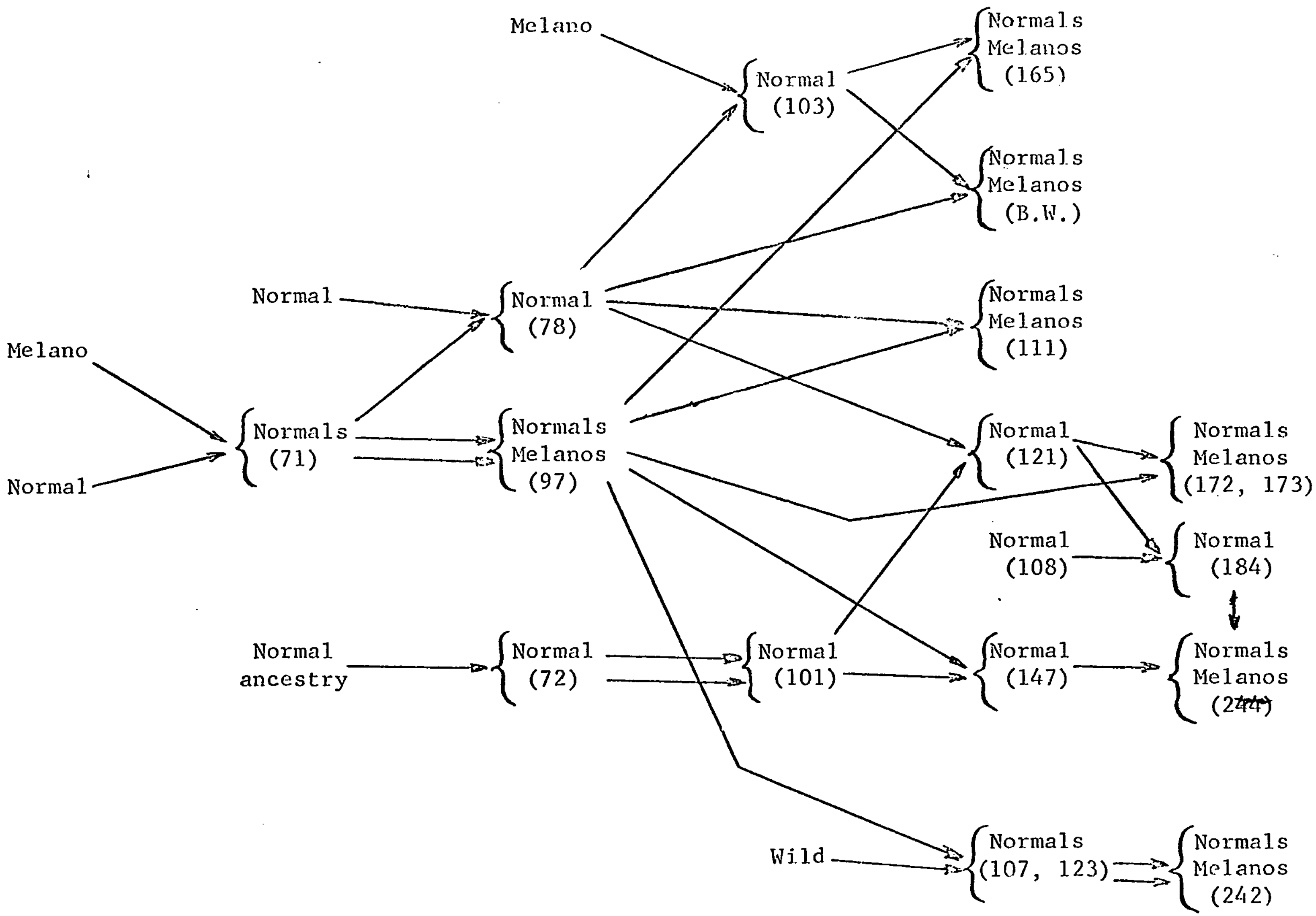


Figure 18. Pedigree chart showing origins and relationships of melanotic Bettas (melanos)

Table 16. Spawns from a normal male (F_1) X normal females with no melanism in their ancestry

Spawn Number	Parents Male	Female	Progeny	
	Normal	Normal	Normal	Melano
76	"	"	95	0
78	"	"	333	0
Total			428	0

Table 17. Spawn from a normal male (F_1) with a normal female that had melanistic fish in her ancestry

Spawn Number	Parents Male	Female	Progeny	
	Normal	Normal	Normal	Melano
205	"	"	47	0

Tables 18, 19, 20, and 21 contain data from matings either expected to produce, or having the possibility of producing, melanistic type in the progeny.

Table 18. Spawns from F_1 males X F_1 females (Melanistic fathers)

Spawn Number	Parents Male	Female	Progeny	
	Normal	Normal	Normal	Melano
97 (F_2)	"	"	49	23
162 (F_2)	"	"	2	0
242 (F_2)	"	"	24	7
Totals			75	30

Expected: 3:1 78.75:26.25
Observed: 75:30
Chi-square .7145 df = 1 P = 50>25%

Table 19. Spawns from melanistic males X normal females, (F_1) reciprocals not possible because of females' sterility

Spawn Number	Parents Males	Females	Progeny	
	Melano	Normal	Normal	Melano
DC	"	"	71	59*
165	"	"	55	33
Total			126	92

Expected: 1:1 (109:109)
Observed: 126:92
Chi-square = 5.302 df = 1 F = 2.5 > 1% (Significant)
* Information from another breeder

Table 20. Spawns from normal males (with melanism in the ancestry) X normal (F_2) females

Spawn Number	Parents Male	Female	Progeny	
	Normal	Normal	Normal	Melano
Spawns lacking melanistic progeny				
199	"	"	44	0
208	"	"	38	0
Totals			82	0
Observed: All normal (if males were homozygous)				
Spawns containing melanistic progeny				
202B	"	"	25	11

One spawn
Expected: 3:1 (if male was heterozygous) (27:9)
Observed: 25:11
Chi-square .592 df = 1 P = 50 > 25%

Table 21. Spawns from normal males X normal females, all fish having melanism in their ancestry

Spawn Number	Parents Male	Female	Progeny	
	Normal	Normal	Normal	Melano
Spawns lacking melanistic progeny				
110A	"	"	74	0
111	"	"	27	0
116	"	"	35	0
117	"	"	68	0
146	"	"	41	0
172	"	"	39	0
178	"	"	50	0
209	"	"	42	0
210	"	"	32	0
216	"	"	13	0
218	"	"	8	0
219	"	"	24	0
225	"	"	5	0
243	"	"	49	0
Totals			507	0
Spawns containing melanistic progeny				
173	"	"	37	6
226	"	"	20	7
244	"	"	42	14
Totals			99	27

Expected: 3:1 (94.5:31.5) if both were heterozygous
Observed: 99:27
Chi-square = .856 df = 1 P = 50>25%

Finally, 588 normal, and no melanistic, progeny were obtained from 16 matings of normal males (with no melanism in the ancestry) X normal females (having melanos in the ancestry).

All results are consistent with the hypothesis of a single mutant, recessive to normal. The physiological mechanism has not been determined.

With the exception of one mating combination, spawns have yielded satisfactory statistical ratios; therefore the exception is attributed to chance. However, a slight bias in favor of the normal allele is suggested. Seven of the nine spawns which contained melanistic classes had more normals and less melanos than the statistical expectations. More information is necessary but these results, in addition to the sterility of melanistic females, indicate a possibility of lower viability of the melanistic type.

At present, breeders refer to melanistic fish as black. The symbol b has been assigned to blond and Bl to blue, therefore another "b" symbol would be confusing. The m proposed by Umrath (1939) for the blond allele has never subsequently been used. Therefore I propose that m (for melanistic or melano) be assigned to the dark form since it more accurately describes this mutant and eleminates the need for a third b symbol.

Most melanistic Bettas look brownish to some degree though some may be almost black. In their "best" color red does not show, however they have been observed to either "lose" dark pigment, allowing red to show, or to change from black to reddish coloration. Occasionally patches of bright red color appear on the fins. Observations on the pigment cells of this type will be presented later.

The availability of a melanistic mutation provides new opportunities for study of the physiology of dark pigment in fishes. In *Betta splendens* there are now mutants which increase (melano), decrease (blond), and almost entirely remove (Cambodia) melanin. Also, the effects of interaction with other color mutants or antagonism between those affecting

melanin may be useful targets of research. It is not yet known, for example, how a fish homozygous for both m and c might look.

Variations in Red Coloration

Red pigment in wild Bettas is limited to portions of the caudal, anal, and pelvic fins and a patch on the operculum. It is much more obvious in males. In domestic stocks, I found it to deviate in three distinct ways: a total absence, an extension to include the entire fish, and a variegated or "piebald" effect. These were observed in various combinations with other color elements. In some combinations red may be hidden.

Absence of red

A series of color "mutants" turned up that superficially did not, at first, appear related (Figures 19 and 20). Various interaction effects, along with their numerous common names, were confusing the issue. The lack of noticeable red color was a single common deviation. I refer to this as "non-red". It should be added, however, that occasionally, in older fish small red flecks or streaks of red appeared.

The non-red individuals can be divided into two major groups, those which have fair amounts of dark pigment and those which do not (Cambodia). Fanciers call the light non-red fish "yellow", "white", or other names, depending on other factors in their makeup. Dark non-red fish are called "brown", "bronze", and "black-yellow", among others, as attempts are made to give them appropriate descriptive identification.

Figure 19. Light (Cambodia) phenotypes of the "non-red" class

a. A "pale yellow" non-red male with a lavender cast produced by a light layer of blue iridocyte color. More yellow than normal.

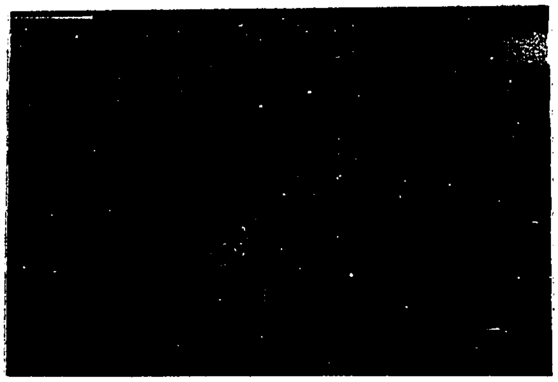
b. Another "pale yellow" male, this one having green iridocyte color.

c. A "yellow" female, non-red, with increased yellow and few iridoctyes.

d. A "pale yellow" male showing the limited amount of dark pigment which can occasionally develop on Cambodia phenotypes.

e. Another "yellow" male, non-red with increased yellow. The limited steel blue iridocyte produces a silvery sheen.

a 

 b

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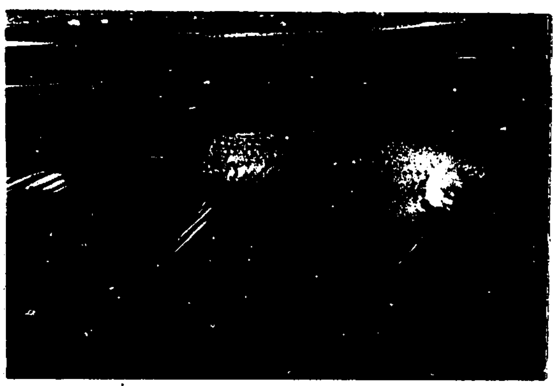
d 

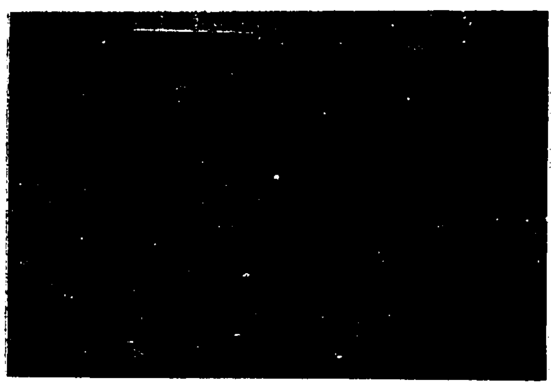
 e

Figure 20. Dark phenotype of the non-red class contrasted with an example with normal red

a. A male possessing red pigment for comparison with non-reds.

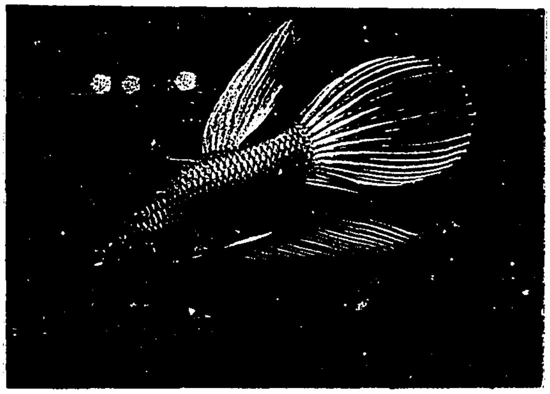
b. A male with wild type coloration except for non-red. Yellowish is visible where red normally occurs.

c. A dark, low sheen, blue, non-red male. The limited spread of iridocyte color allows the non-red to show, especially on the fins.

d. A similar male with green iridocyte color, photographed against a dark background to show altered appearance. Non-reds are best classified against light backgrounds. The red visible below the head is on the protruded gill membrane rather than part of the external integument.

e. A dark, spread steel blue, non-red male. Creamy yellow color is visible (especially on the pelvic and anal fins) where red normally appears.

f. A dark, spread blue, non-red male, similar to the last except for the iridocyte color.



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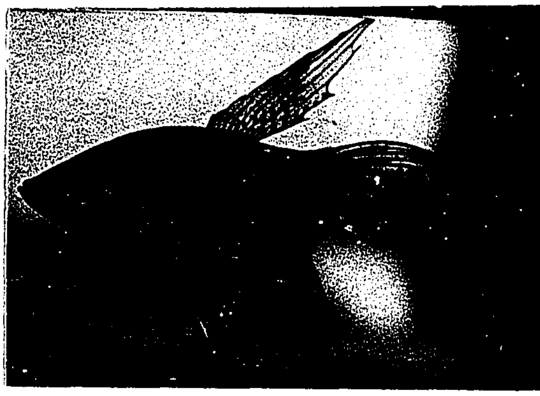

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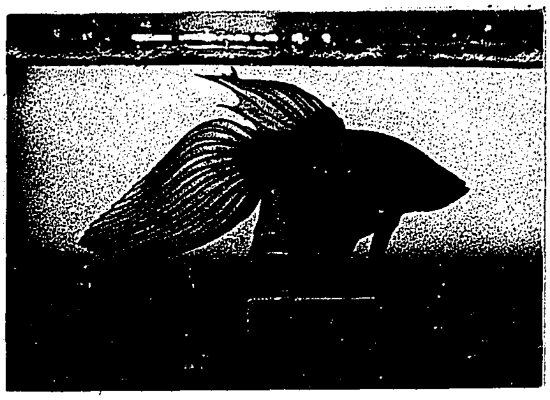

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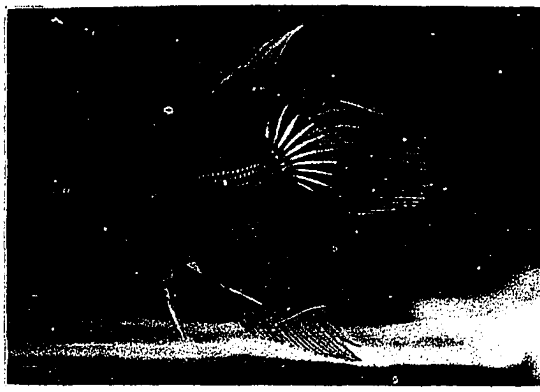

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Figure 21 is a pedigree chart of matings involving non-reds. A considerable number of spawns could be classified for the condition.

Five non-red X non-red matings produced all non-red progeny (Table 22); therefore the abnormality breeds true.

Table 22. Spawns from non-red males X non-red females

Spawn Number	Parents Male	Female	Progeny	
	Non-red	Non-red	Normal	Non-red
Dark non-reds				
108	"	"	0	100 plus
167	"	"	0	40 plus
Cambodia non-reds				
180	"	"	0	60 "
240	"	"	0	5
248	"	"	0	70 "
Total			0	275 "

Sixty-seven spawns from normal X normal (with no known non-red in the ancestry) yielded 4,639 progeny, all normal. Other breeding data involving non-red are given in Tables 23, 24, and 25.

Only non-red females were used in outcrosses (Table 23). The results clearly show that non-red is recessive. The progeny in F_2 and test-cross spawns occurred in proper classes and ratios to support the hypothesis of a mutant gene at a new locus which prevents the normal development of red pigment.

Though progeny numbers are low, F_2 spawn 232 is of special

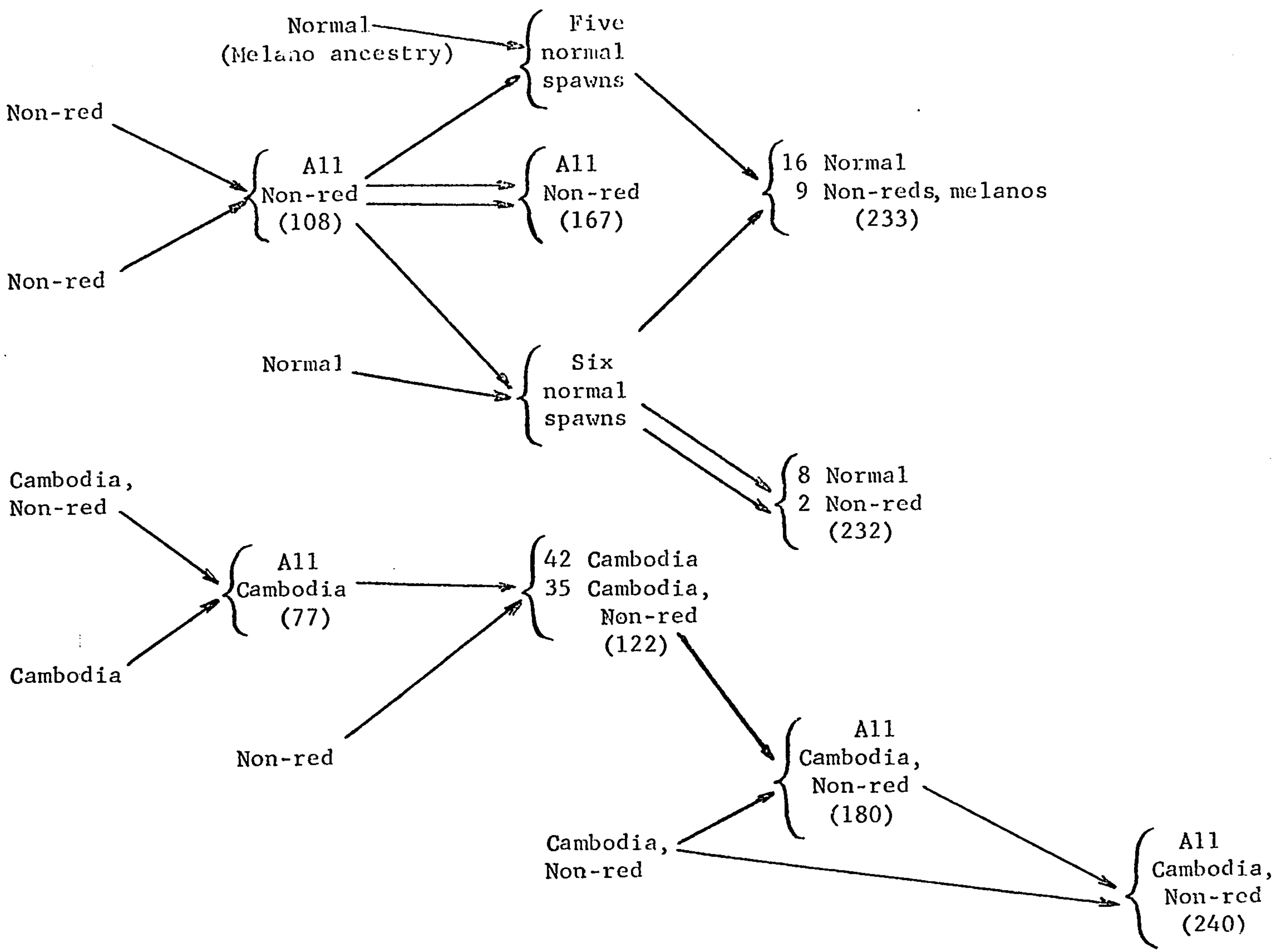


Figure 21. Pedigree chart showing origins and relationships of "non-red" Bettas

Table 23. Spawns from normal (having no non-red ancestry) male X non-red female matings

Spawn Number	Parents Males	Parents Females	Progeny Normal	Progeny Non-red
	Normal	Non-red	Normal	Non-red
168B	"(Dark)	" (Dark)	36	0
171	" "	" "	20	0
184	" "	" "	48	0
185	" "	" "	50	0
186A	" "	" "	44	0
189	" "	" "	25	0
Sub-total			223	0
77	"(Cambodia)	"(Cambodia)	188	0
100A	" "	" (Dark)	33	0
174	" (Dark)	" "	48	0
175	" "	" "	27	0
Sub-total			296	0
Total			519	0

Table 24. F_2 spawns from matings where both parents were F_1 of normal X non-red

Spawn Number	Parents Male	Parents Females	Progeny Normal	Progeny Non-red
	Normal	Normal	Normal	Non-red
226	"	"	19	8
232	"	"	8	2
233	"	"	16	9
244	"	"	42	14
Totals			85	33

Expected ratio 3:1 (88.5: 29.5)
Observed: 85:33
Chi-square = .554 df = 1 P = 50>25%

Table 25. Test cross spawn from normal male (F_1 from non-red father) X non-red female

Spawn Number	Parents Male	Female	Progeny Normal	Non-red
	Normal	Non-red	Normal	Non-red
122	"	"	42	35

Expected ratio: 1:1 or 38.5: 38.5
Observed 42:35
Chi-square = .636 df = 1 P = 50>25%

significance, (Table 26). It was produced by two non-Cambodia fish from F_1 spawn 189 which also contained Cambodias.

Table 26. F_2 progeny in spawn 232 showing recombination of non-red with Cambodia

Parents Male	Female	Progeny Normal	Cambodia	Non-red	Cambodia Non-red
Dark Red	Dark Red	Normal	Cambodia	Non-red	Cambodia Non-red
#189-1	#189-2	6	2	1	1
Expected: (9:3:3:1)		5.625	1.875	1.875	0.625
Individual Chi-square		.025	.0085	.408	.2256
Chi-square = .6671	df = 3	P = 90>75%			

The phenotypic classes in spawn 232 fit the 9:3:3:1 ratio adaquately. These data imply that no linkage exists between the non-red and Cambodia loci.

Since no previous mention has been made of this abnormality there is no symbol priority to consider. I therefore propose the symbol <u>nr</u>,

for non-red, for this mutant. The red flecks found in some homozygous non-reds (discussed with yellow) may be somatic mutation effects.

Variegated or "Piebald" red ("Butterflies")

A blotchy appearance caused by the uncommon loss of pigment after it has developed will be considered later as an apparently non-genetic phenomenon. Red variegation, by contrast, tends to be stable and limited to the fins.

The effect produced in red variegated phenotypes is often quite striking (Figure 22). For this reason hobbyists have been calling them "Butterfly" Bettas. Breeders usually select fish which are also Cambodia and with minimal sheen for maximum color contrasts. Red fails to develop in some areas, usually in distal portions of pigmented fins. In some instances the proximal portions are affected. In more extreme cases the color is irregular or may be totally absent, though the latter type is quite rare. Demarcation lines between red and non-red areas are usually abrupt. Red is the last color to be seen on developing young Bettas and they should not be classified before it is plainly visible. Once patterns are established on specific fish they do not change other than to expand with fin growth.

Matings between non-variegated parents having no variegated types in the known ancestry bred true without exception: I had 54 spawns containing 3,022 progeny, none variegated. The same types crossed with fish that were classified normal though from variegated stock produced some variegated though it happened that these crosses also involved an extended red

Figure 22. Some variations in variegated Bettas

a. Variegation in an otherwise normal-colored male.

b. A variegated male in darker display color.

c. A blond (_bb_) variegated male with green iridocyte color.

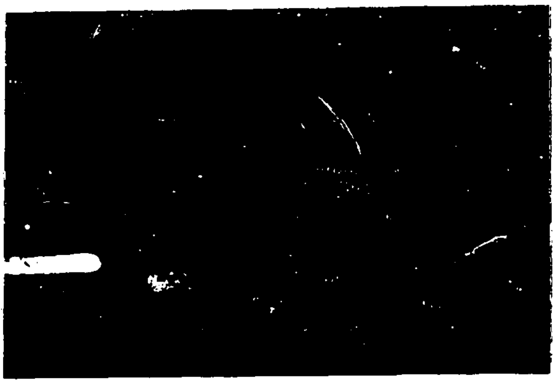
d. A Cambodia (_cc_) variegated male with steel blue iridocyte color.

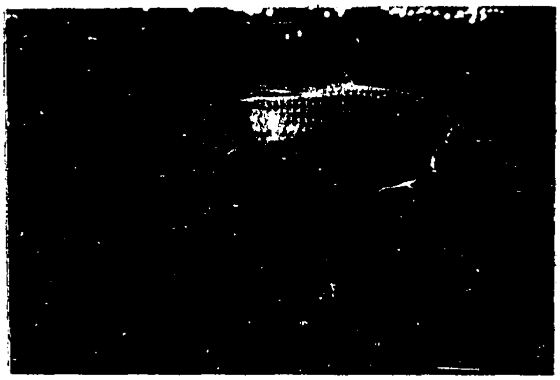
e. A Cambodia (_cc_) variegated male with more extensive red in fins.

a 

 b

c 

d 

 e

abnormality which may have counterbalanced the variegating effect. In all other mating combinations involving variegated fish or fish with variegated ancestry some variegated types were obtained (Table 27).

The range of variegation is shown in the series of photographs of sibling males from spawn 74 (Figure 23). Classification into major groups is not difficult if fish with fins entirely covered by red are considered normal (caudal and anal fins) and those having white or transparent areas in place of some or all of the red are considered variegated.

Two spawns (74 and 77) were sub-classified for type of variegation (Table 28).

Though spawn 74 was obtained from two extremely variegated (nearly colorless) individuals from spawn 45 there were all classes of progeny including normal. Since only one mating failed to contain normals and the progeny number was quite low (15) it is probably reasonable to infer that variegated X variegated crosses do not breed true. A pedigree chart of variegated matings is provided (Figure 24).

The rare occurrence of seemingly colorless individuals is significant in considering the possibility of non-reds as having been selected from extreme variegated stocks. Obviously, individuals with extreme limitation of red in their own phenotype are still able to produce progeny with essentially normal red color.

Wallbrunn (1951) discussed the development of clear borders and colorless fins and so must have worked with this same abnormality. He presented no data but did state "The gene (or genes) which eliminates red in the fins has variable penetrance and expressivity". My breeding data

Table 27. Spawns from matings in some way involving variegation

Spawn Number	Parents: Males	Parents: Females	Progeny: Normal	Progeny: Variegated
	Normal*	Normal**		
72	"	"	206	?***
84	"	"	10	"
100	"	"	19	"
114	"	"	60	Some
115	"	"	57	?***
120	"	"	54	"
121	"	"	64	Some
137	"	"	7	?***
148	"	"	15	"
159	"	"	9	"
Sub-totals			501	Unknown
	Variegated	Variegated		
74	"	"	23	89
LW-3	"	"	0	15
LW-10	"	"	13	25
198	"	"	Some	Some
264	"	"	Some	Some
Sub-totals			36 plus	129 plus
	Normal**	Variegated		
LW-2	"	"	16	50
201	"****	"	Some	Some
45	Variegated	Normal*	424	152
77	Non-red*	Variegated	129	59
122	Normal**	Non-red*	14	28

* No known variegated in pedigree but often from outside stock.

** Pedigree includes variegated

*** Pedigrees include extended red types. Progeny were not classified for variegated though some are known to have had it.

**** Normal males from outside stock. Source was known to be working with variegated stocks.

Figure 23. A selection of siblings from spawn 74 showing range of red variegation. They are Cambodia (cc) type and also exhibit variation in iridocyte color

a. A spread green male showing the extent to which it is possible to obscure the deeper colors. It is impossible to determine in this picture the extent of red. The fins must be lighted from behind for classification.

b. A male with red classified as normal.

c. Slight limitation of red, mostly visible along the proximal and distal margins of the medial fins.

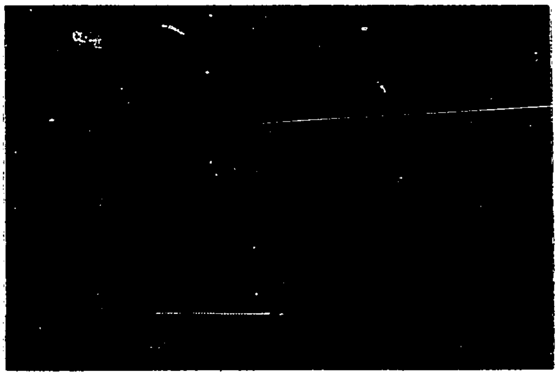
d. A more noticeable limitation of red, partially hidden by the denser iridocyte color.

e. More limitation, both proximal and distal in the fins.

f. More limitation.

g. More limitation with the general range interrupted so that color does not appear to be present al all through large areas.

h. A male with no visible red pigment, phenotypically, but not genetically, like a non-red.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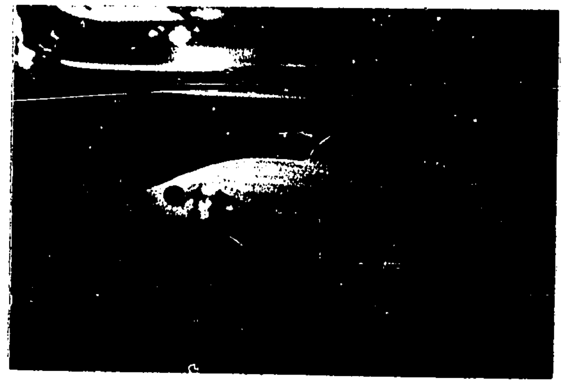
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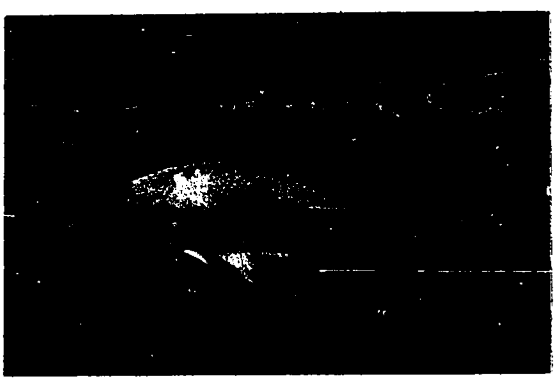
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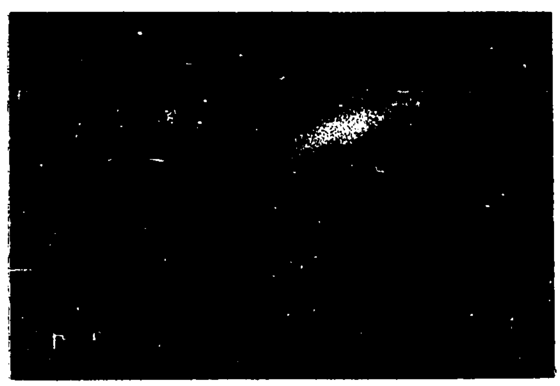
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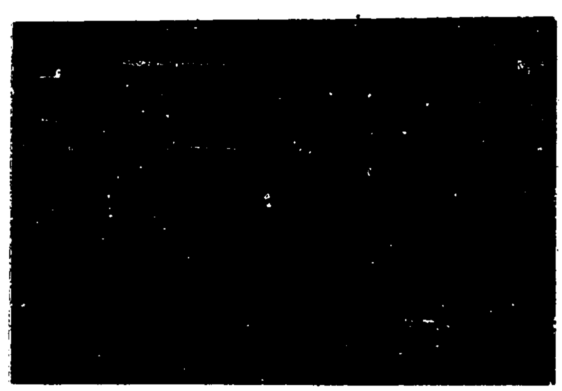
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Table 28. Breakdown of two homozygous Cambodia spawns into sub-groups of variegation in the fins

	Color Groups					
Spawn Number	"Normal"	Proximal limitation	Distal limitation	Both	Irregular limitation	No Red
#74 (males only)	23	38	9	11	32	1
77						
males	40	8	6	0	4	0
females	89	33	0	0	8	0
Sub-totals of #77	129	41	6	0	12	0
Totals	152	79	15	11	44	1

indicate a genetic basis for variegation, probably a dominant mutant, expressed as indicated by Wallbrunn.

There may be antagonism between this partial limitation of red and over-production in the extended red type (to be discussed next). Also, the data do not preclude the possibility of variegated being homozygous lethal. Though additional information is required, I propose the tentative symbol Vf signifying variegated fins. While fanciers use the term "Butterfly" extensively, the name is not descriptive and its initial would be confused with those of blue and blond. V is also undesirable since it was previously used for iridocyte color.

Abnormal extension of red

Another popular Betta color is an intense red. By comparison with

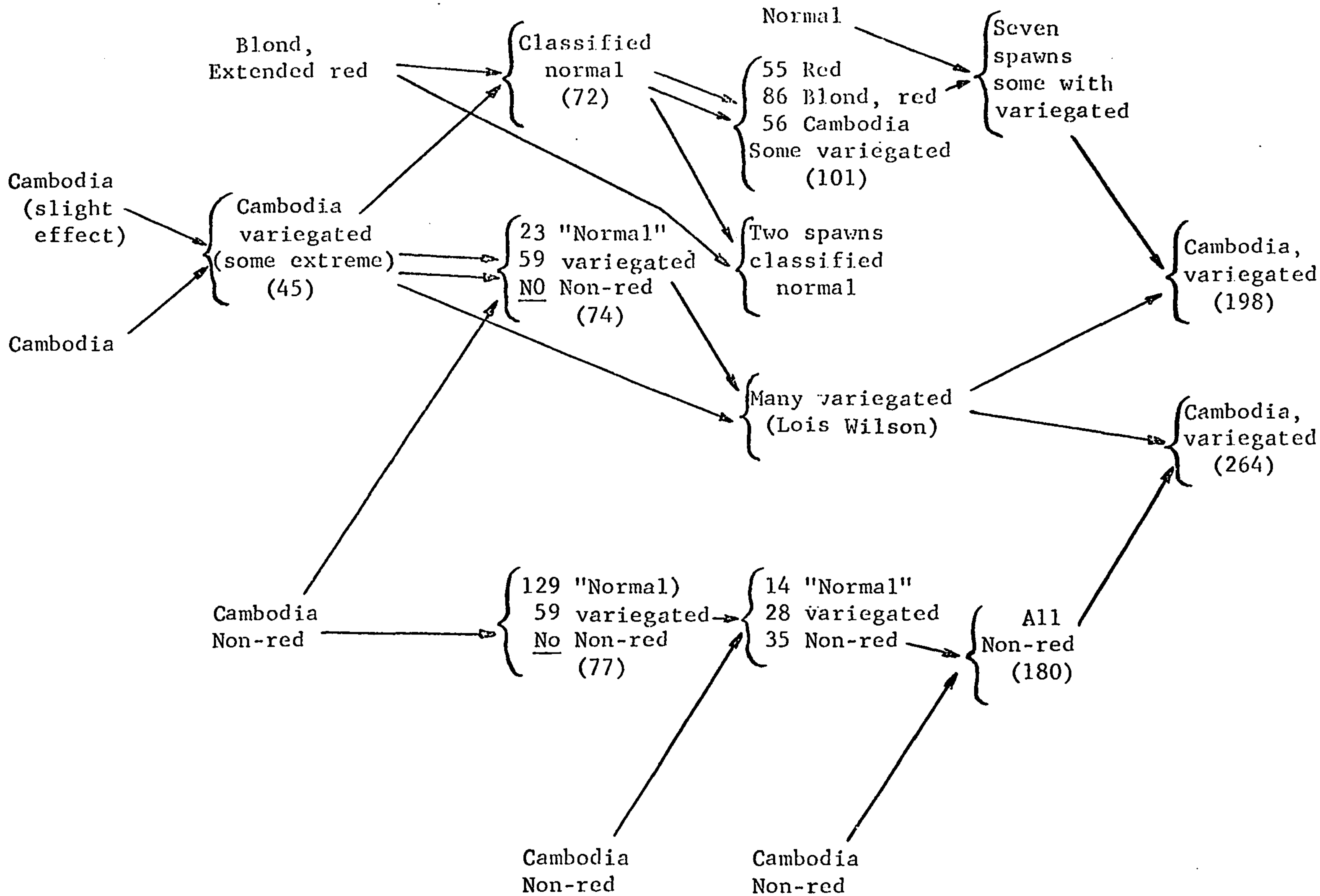


Figure 24. Pedigree chart of matings involving variegated Bettas

normal red distribution the phenotypes have intense red pigment visible over most of the body as well as red fins. Since wild fish have no red cells on body scales the presence of this intense red color is decidedly abnormal. I am using the term "Extended red" to refer to all red fish.

The presence of extended red in combination with the many other color variations produces an additonal class of female types (Figure 25). Breeders distinguish between them in a non-elemental sense, giving them names like "blood red", "tomato red", "bright red", "lavender", and "purple".

As shown in the breeding chart (Figure 26) and in Table 29, extended red fish almost always produced red progeny.

Twelve spawns from parents with no known red ancestry produced only normal colors in 802 progeny.

A series of matings of sibling males from an inbred red strain crossed to sibling females from a normal line produced all red progeny (Table 30).

From data presented it appears that red can breed true, normal breeds true, and red X normal crosses yield only red progeny. A dominant mutant is implied. However, exceptional results were obtained from red X non-red crosses (Table 31) and fish of either type having melano and (or) non-red ancestry (Table 32).

The exceptional results may be due to antagonism between red and other mutants. Additional information is required to clarify this interaction. The data from other tests seem adequately in support of the idea of a dominant mutant which somehow either greatly increases the formation of red pigment or alters other pigment cells.

Figure 25. Extended Red Bettas compared with normal

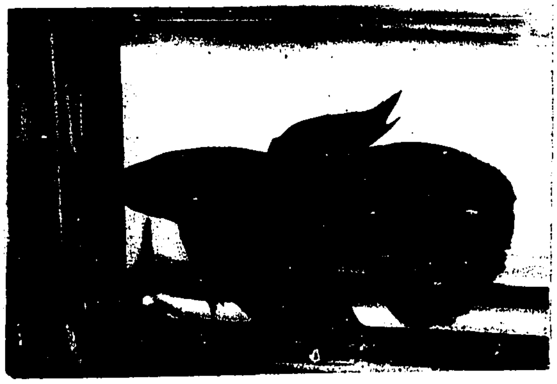
a. A male classified as normal for red. Red pigment is limited to the fins and the normal locations on the body.

b. A red male. Red is visible on the body, especially on the dorsal area of the head.

c. A blond, red male. The dark pigment shows in spots on the scales but check areas are quite light compared with darker fish.

d. A Cambodia, red male. Red on body with over-lying blue iridocyte color creates a "lavender" color effect.

e. A red male with the same blue iridocyte color overlay. Against the dark background the effect is "purple". Again the red is very evident on the dorsal surface.

a 

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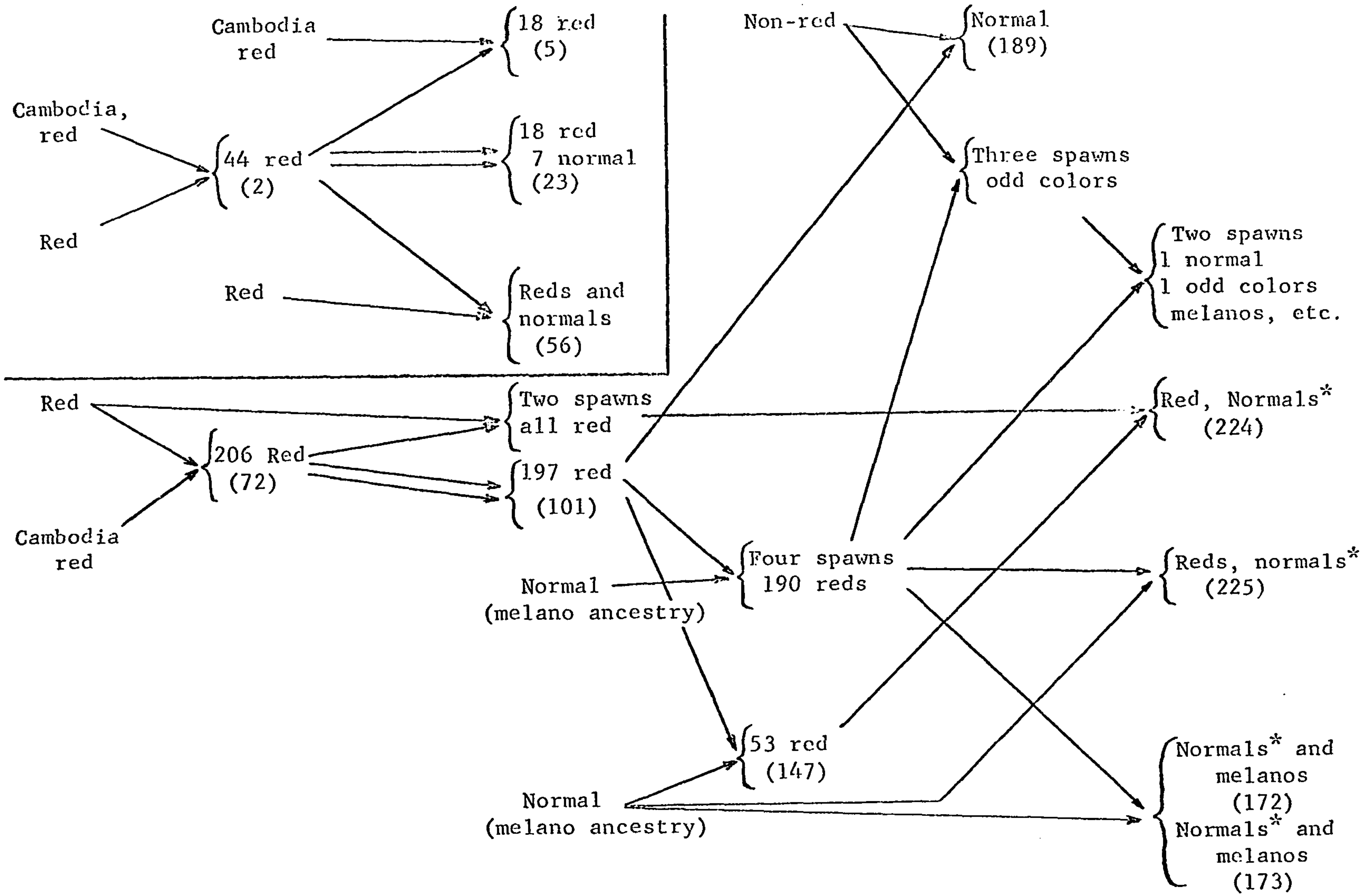


Figure 26. Pedigree chart showing origins and relationships of Extended Red Bettas
*Fish looked abnormal but not exactly red

Table 29. Spawns from red X red matings (parents from various sources)

Spawn Numbers	Parents Male	Female	Progeny	
Spawns producing only red progeny	Red	Red	Normal	Red
61	"	"	0	146
84	"	"	0	10
100	"	"	0	19
101	"	"	0	197
Sub-total			0	372
2	Red Cambodia	"	0	44
5	"	"	0	18
Sub total			0	62
72	Red	Red Cambodia	0	206
Totals				640
Spawns producing both red and normal progeny				
23 (F_2)	Red (F_1 of sp.2)	Red (F_1 of sp.2)	7	18
225 (F_2)*	(" " " 121)	(" " " 147)	2	3
Totals			9	21

Expected: 3:1 (22.5: 7.5)
Observed: 21:9
Chi-square, with Yate's correction for low numbers = .177 df = 1
P = 75>50%

* Spawn 225 constitutes a classical F_2 except that F_1 parents were not siblings. Both were F_1 from normal X red cross.

Table 30. Spawns from red (inbred strain) male X normal females with no known red ancestry

Spawn Number	Parents: Male	Parents: Female	Progeny: Normal	Progeny: Red
	Red	Normal	Normal	Red
114	" (sp. 101)	" (sp. 78)	0	60
115	" "	" "	0	57
121	" "	" "	0	64
147	" "	" "	0	53
159	" "	" "	0	9
Total				243

Table 31. Spawns from Red males (two origins) X non-red females

Spawn Number	Parents: Male	Parents: Female	Progeny: Normal	Progeny: Red
			Normal	Red
184	Red (sp. 121)	Non-red (sp. 108)	48*	0
185	" "	" " "	50*	0
171	" "	" " "	15*	0
189	" (sp. 101)	" " "	25*	0
Totals			138	0

* Fish generally looked normal with areas "trying" to look reddish and (or) blackish. Not quite normal.

Table 32. Unusual spawns from red X red or red X normal matings in which one or both parents have melano and (or) non-red ancestry

Spawn Number	Parents: Male Color	Male Ancestry	Female Color	Female Ancestry	Progeny
173	Red (sp. 121)	(melano)	Normal (sp. 97)	(melano)	42 progeny. 5 melanos, others appear normal.
224	Red (sp. 100)		Red (sp. 147)	(melano)	26 progeny. 23 yellowish-red, 1 red, 1 variegated, 1 almost non-red.
226	Red (F_1 of sp. 147)	(melano)	Red (F_1 of sp. 147)	(melano)	27 progeny. 19 appear normal, several shades of melano, 1 "white" with gray smudges in fins (Cambodia possible).
244	Red (sp. 147)	(melano)	"Normal" (sp. 184)	(melano, non-red)	56 progeny. 42 appear normal. Melanos of many shades, some reddish, 1 "mustard" color with black fins.

Wallbrunn (1951) suggested that at least two genes, one affecting the body and the other the fins are involved. Of these he said "The gene (or genes) which produces red on the body of male Cambodias is not recessive". Neither he nor Umrath (1939) compared the pigment in red phenotypes with that in normal (wild type) fish. Neither suggested a symbol for extended red or whatever they were describing that correspondes to it. I suggest the letter R (for red) which has not been used for any Betta mutant, not to be confused with Eberhardts' Ri (that I have abandoned which was used to describe iridocyte distribution). The relationships of red variations to wild type are diagrammed in Figure 27.

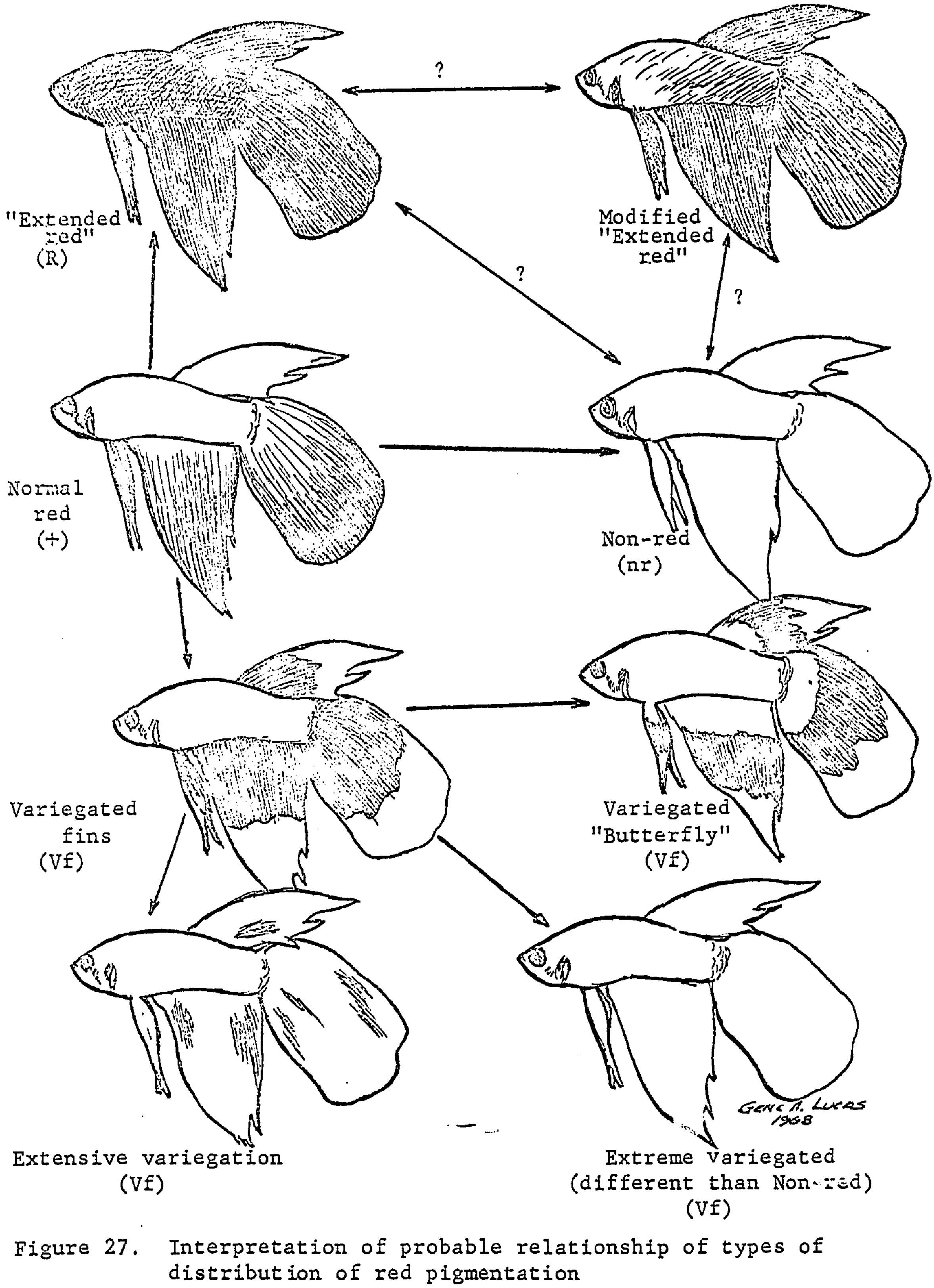


Figure 27. Interpretation of probable relationship of types of distribution of red pigmentation

Variations in Yellow Coloration

Yellow pigment in wild fish is not superficially obvious, although there are nearly as many xanthophores as melanophores in the scales. Some domestic phenotypes are, however, noticeably yellow. These have been shown in Figures 19 (p. 124) and 20 (p. 126).

The first mating in this series was made with a yellow male (from outside stocks) X a light Cambodia from a variegated line. The male was not at that time recognized as a Cambodia and non-red. The progeny from this cross were all Cambodia (spawn 77). A second yellow (a female) was obtained and mated to an F_1 male. This mating produced 42 Cambodias and 35 "yellow" (spawn 122). A third yellow (a male) was obtained from outside stock and when it was mated to a yellow from spawn 122 all yellow progeny were obtained (spawn 180). The same male was then mated to one of his daughters and again all yellows were obtained (spawn 240).

An initial problem was failure to recognize that the yellow of "yellow" phenotypes depended upon the absence of black, the absence of red, and then the increase of yellow. The possibility of an independent yellow pigment which could be present or absent on Cambodia bodies was not discovered until the more blatant pigment elements were understood.

The variation from pinkish to yellow color is not easily subgrouped in either Cambodia or dark fish. This contrast may be observed in Figure 19-c (yellow) and Figure 23-h ("pink") but few individuals are as extreme. Yellow color was not apparent in spawn 77 progeny until many had been disposed of. Later a number of them became very yellow while others resembled ordinary Cambodias.

No reliable method of classification was developed but scrutiny of many fish led to the conclusion that "yellow" may in fact be an alteration of red. It is possible that a synthetic pathway in non-reds could be blocked, leaving an intermediary (yellow). Fish that were Cambodia and non-red had yellow in the body areas ordinarily red. A number of non-Cambodia non-reds were "mustard" colored, while others were very dark. These odd-colored fish had reduced black (blond) and extensive yellow distributed over the head and to other areas where red pigment is found in extended red examples. Also some Cambodia fish with red fins had extensive yellow coloring of the head and body. It may be that Wallbrunn's suggestion of two genes controlling the development of red, (one on the body, the other on the fins) is a lead. Possibly there is not just one pigment involved. Thus one could be absent and the other changed to yellow! At least there is strong evidence that the phenotype yellow is highly dependent upon the red genotype.

As noted in connection with non-red, on many occasions small flecks of red developed in otherwise yellowish fins of both Cambodia and non-Cambodia. One perplexing blond fish with brilliant red fins had red on the lower part of the body grading into a mustard colored dorsal half.

At present it is impossible to say whether a separate "yellow" mutant exists or whether the yellow phenotypes are all interactions of recognized mutants such as <u>nr</u>, <u>b</u>, <u>R</u>, and <u>c</u>.

An "Opaque" Variation

In 1966 I obtained some unusual Bettas from Mr. Walt Maurus of Livonia, Michigan (Figure 28). Their origin is unknown. They have deposits of some unidentified material, probably guanine, throughout the integument, best observed in mature fish. It produces a pinkish-white to yellowish-white color which seems, in part, to intermix with, and partially to cover the ordinary color. The effect is as though the fish had been dropped in cream-colored paint and was poorly cleaned.

The deposits are similar (possibly identical) to the deposits in the peritoneum and other ventral areas on normal fish. If the substance is guanine, at least microscopically it does not look the same as that of the "crystals" which produce iridocyte color. Neither is it limited to the same areas nor does it replace iridocyte color.

Both materials exist in the same individual and iridocyte colors appear normal but partly hidden. Because of the masking property, I refer to the condition as "opaque".

A pleiotropic effect of this mutant seems to be the occasional enlargement and protrusion of one or both eyes. Eye conditons of this nature are found in other color types but they seem to be more frequent in opaque fish.

A pure stock was being maintained by Maurus which had Cambodia and spread green iridocyte color in all individuals. As a result, the breeder was seeing only one phenotype which he referred to as "Bronze". This name is not appropriate. The real abnormality, if combined with another phenotype such as steel blue, could produce a different color effect.

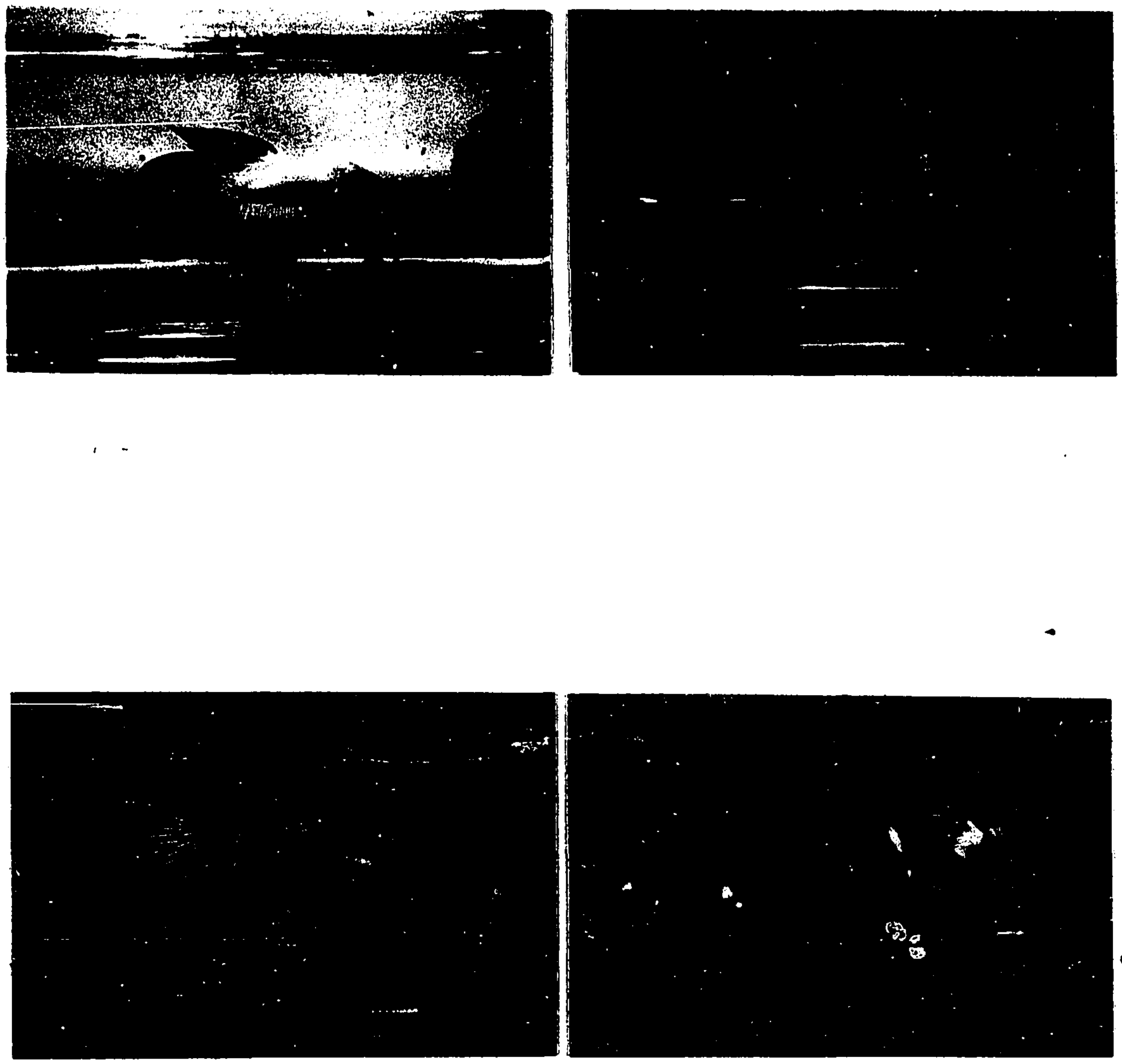
Figure 28. The "Opaque" abnormality

a. An "opaque" male. A Cambodia with green iridocyte color. Note altered appearance of colors, also "white" head area and enlarged eye.

b. An "opaque" female of the same color type.

c. A young F_2 male with a considerable amount of "opaque" pigment. Eye slightly affected.

d. An "opaque" Cambodia (left) compared with a "normal" Cambodia. The deposits in the normal fish are in the lower cheek areas and on the operculum. On the "opaque" fish they are spread to all scale areas, especially over the dorsal parts which look more transparent on the ordinary fish. The deposits are even noticeable in the eye.



a b c d

Only a few spawns were obtained from crosses (Figure 29) but inferences may be drawn from the fact that spawn 164, an opaque (Maurus inbred strain) X normal (no opaque in ancestry) cross, produced all opaque progeny, the effect being intermediate. Two F_2 spawns were extremely small (two and five individuals, respectively) yet each produced at least one normal and one Opaque. More data are required but tentively I propose a mutant at a single locus, dominant or partially dominant to normal, and suggest the symbol O (for Opaque).

Color Loss and Albinism

Breeders constantly search for albino Bettas but genetic albino strains have never materialized. The nearest approximation is the "temporary" phenotypic albinos which remain following the extensive loss of pigment in what appear otherwise to be normal fish. There is no uniformity to the progressive changes so individuals are highly variable (Figures 30-a, b, c, d, e, f, g, h; Figure 31-a, b, c, and d).

The occurrence of this abnormality is not very common, but most breeders who raise quantities of Bettas notice them occasionally. I have observed enough of them to be able to describe some of their developmental features.

The first sign of deterioration seems to be lightening in irregular patches as black pigment disappears. This has the most noticeable effect on the color phenotype as the fish may progressively lose it all and become a "synthetic Cambodia", or "albino". If pigment deterioration continues (and it usually does), iridocyte color and red soon follow.

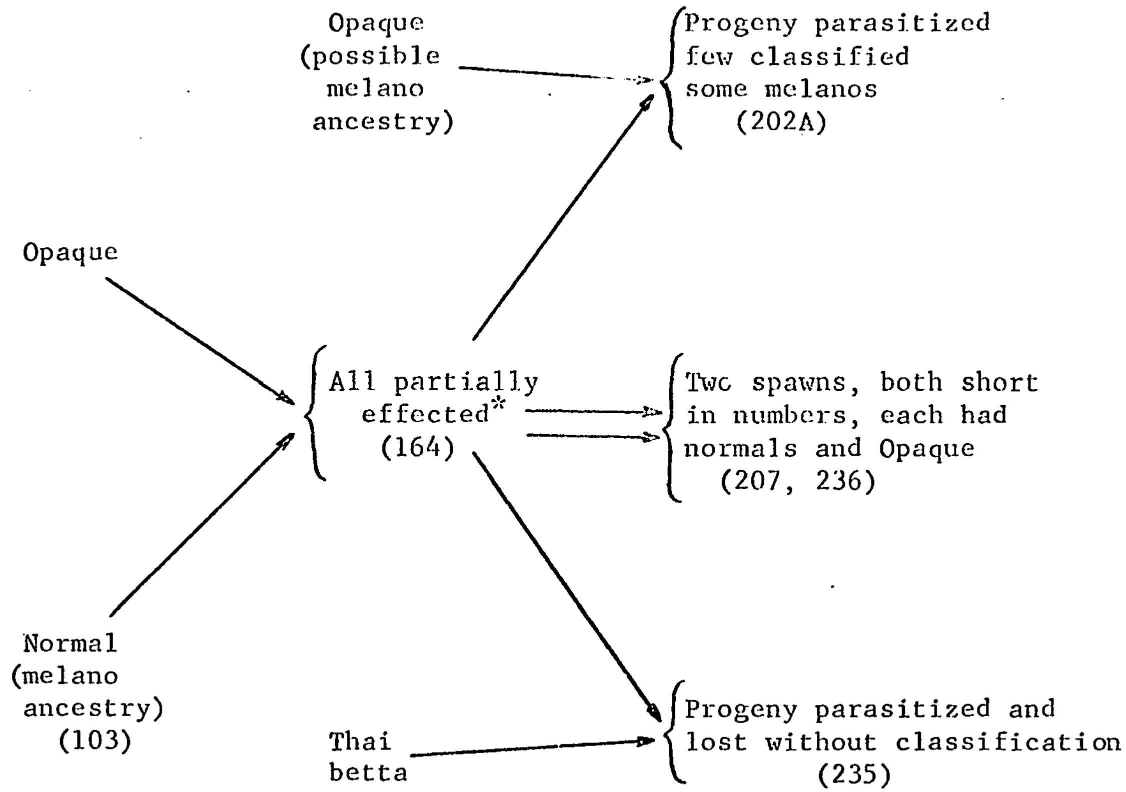


Figure 29. Pedigree chart showing origins and relationships of "Opaque" Bettas

*Moderately affected, seemed to get worse as they got older

Figure 30. Bettas in stages of color loss

a. A formerly purple male which has lost most of its color. Patches of red remain in the anal and caudal fins, the retina is black and the iris of the eye has considerable iridocyte color.

b. The same male about five weeks later showing recovery of some red pigment, no others.

c. A former melano which has lost most of its pigment. Small amounts of black are still visible on the fins, in the eye and on the gill membrane.

d. The same fish a few days later. Color disappeared rapidly, finally even from the retina.

e. A young male moderately affected, black pigment, primarily, is absent.

f. The same fish several weeks later in a "recovery" stage.

g. The same fish still later, when it has regained its normal color, the inside of the eye was pink.

h. Initial stages of color loss. Part of head and one eye involved. Pigment lost from retina giving a "pink" eye.


a


b


c


d


e


f


g

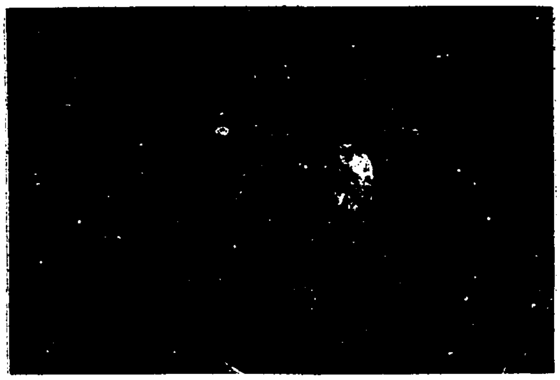

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Figure 31. Partial color loss and "colorless" Bettas

a. A young male with the blotchy appearance first observed when black pigment is lost. Other pigments not yet affected.

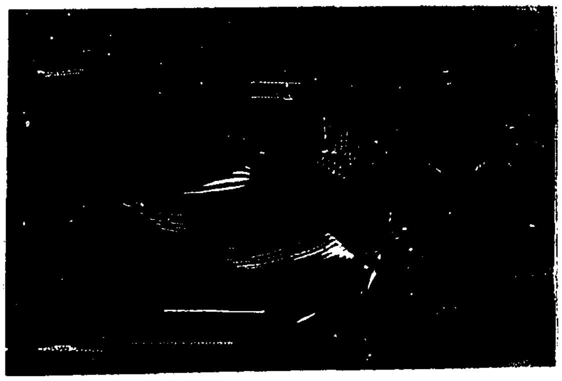
b. A sibling to the first fish in about the same condition Note the Cambodia-like appearance of the light areas.

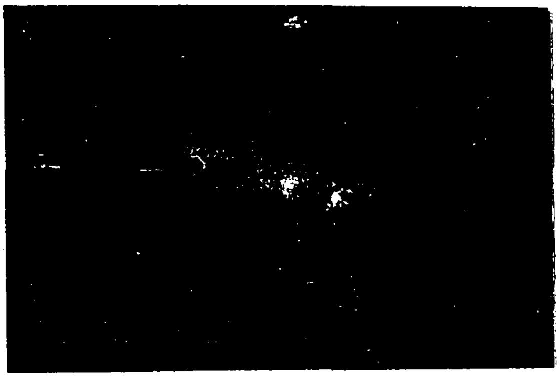
c. An extreme variegated Cambodia female. No yellow, black, or red integumentary pigment is visible. The retina is black and small amounts of green iridocyte color are visible on the fins.

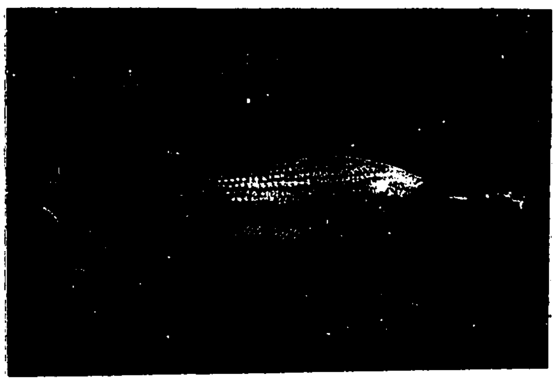
d. A spread, green male, losing black pigment. It is absent from the anal, dorsal, upper caudal, and the central part of the body.

e. A young "white" male which developed as a Cambodia, non-red. No yellow is evident. Iridocyte color is steel blue which looks silvery white on the light background. The retina is black and the iris has some pigmentation.

f. The same fish photographed against a contrasting background to show the "white" of the steel blue iridocyte color.

a   b

c   d

e   f

The process may be completed in a week or it may take several. It may be arrested or even reversed at any time. If color re-forms red develops first (Figure 30-a and b) followed by iridocyte color, then melanin. Possibly because of ontological specialization and separation from general pigment milieu, eye pigments respond slower, being the last to disappear. They also lag in recovery. The fish in Figure 30-e, f, and g, for example, never completely lost its body pigment and recovered almost completely but finally had "albino eyes". This individual, during its color recovery, went through a most striking color phase (Figure 30-f).

The condition must be unrelated to genetic color "lack" (Cambodia, non-red) since those abnormalities are permanent. Those types do develop and retain black choroidal pigment (Figure 31-c, e, and f).

The criterion of eye pigment is important since Cambodias and other mutants with normal retinal pigment always have normal vision whereas individuals losing eye pigment become progressively blind. The fragmentary and apparently destructive nature of eye pigment loss is shown in Figure 32-c and f.

As black pigment disappears the retina becomes redder as expected in albinos. The fish might then be considered "transitory" albinos. The problem of terminology lies with the definition of albinism. Fish fanciers refer to almost any red-eyed fish that is light colored (even yellow or red) as albinos, and the possibility of genetic relationships or relative permanence are given little consideration.

The reason for color loss is unknown. Wallbrunn (personal communication, 1964) thought he could see pigment at the bottom of containers,

Figure 32. "Pink" eyes of Bettas with deficient or degenerating pigment

a. The head and eye of the formerly melanistic fish shown in Figure 29-a and 29-d. At this stage retinal pigment is absent and most is gone from the iris.

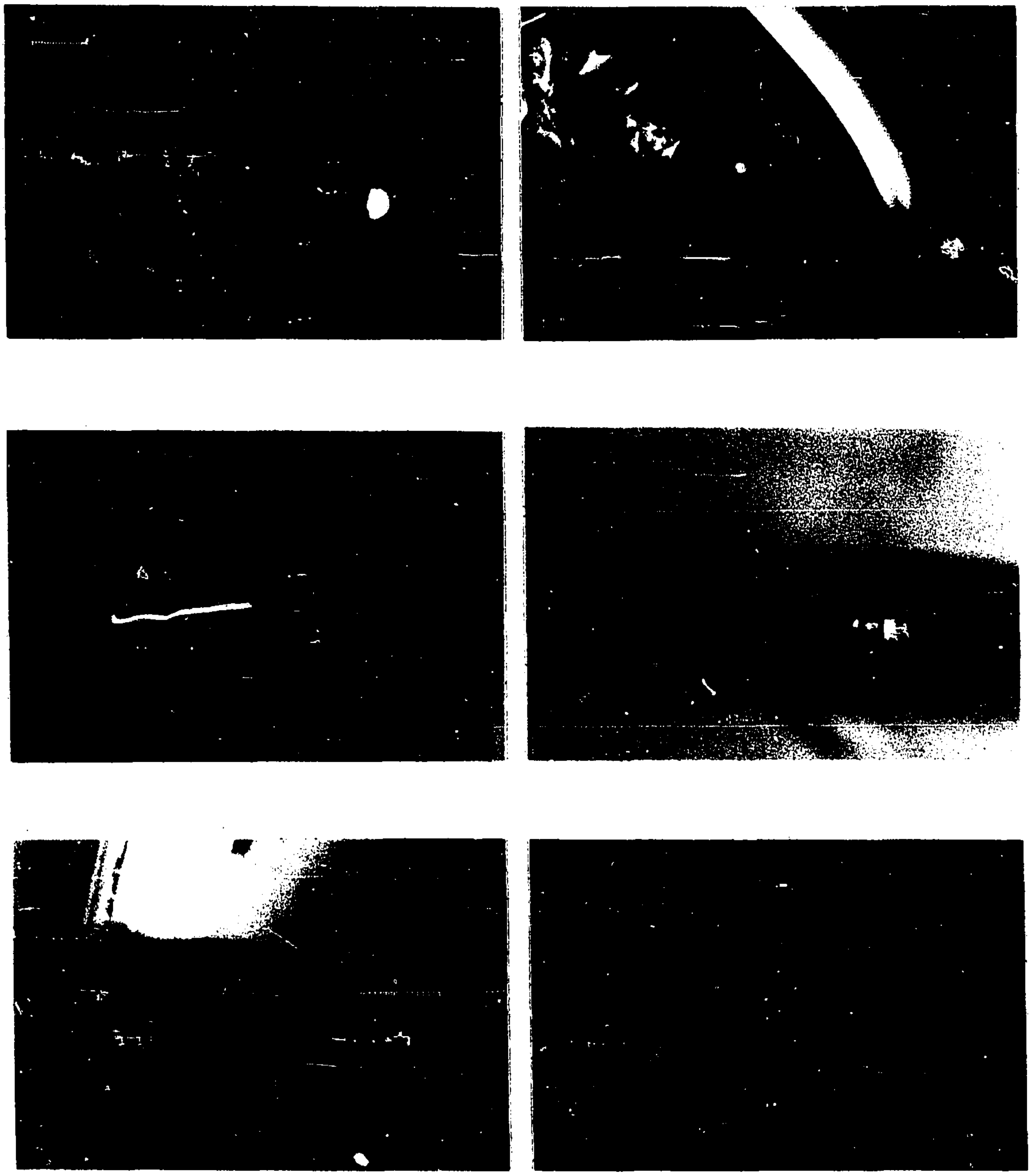
b. The same eye. The unpigmented area includes a pigmentless area of the iris.

c. A formerly red male. Little body or fin color is left. Little retinal pigment (the eye was quite pink though the print does'nt show it) some black remains in the wall of the eye.

d. A pink eyed with a pigmentless retina. Some patches of reddish-yellow were visible on the sides of the fish. Spawn-mates were also variously affected.

e. The eye of the same fish, showing fragmentary black patches in the retina.

f. Another view of the same fish.



a b

c d

e f

but I cannot confirm this. That pigment and (or) possibly pigment cells are absent is evident. A scale from the fish shown in Figure 32-c appears in Figures 7-h, (p.77), 8-h(p.79), and 9-h(p.81). It is clearly devoid of color though unpigmented cells could be present.

There is slight evidence for a hereditary basis. Two spawns (relationship unknown) produced 5 and 2 affected individuals respectively. Also, I have found only males affected so far. The fact that males are cared for individually and are large and showy makes it more likely they would be noticed. An affected female in a population tank might never be.

Attempts to breed these types have been largely negative as they are practically unable to perform spawning duties. I feel they should not be called albinos since they are not, at this time, either a reproducible strain or a permanent color type.

Further Discussion of Color Effects

Pigment cell counts were used in some cases by past workers to differentiate phenotypic classes, but consideration was not given to pigment cell populations in terms of their relation to wild type. It is most useful to include the concept of mutants in the development of "genetical systematics" for any organism. It is unfortunate that wild Betta stocks were not available for most of my genetic tests. Further tests using them will surely clarify some of the more confusing variations, especially those involving antagonistic and synergistic interacting effects. However, the wild-type substitutes have been effective, and the genetic data now

available, and with observations on pigment cell shifts, make phenotypic diversity reasonably comprehensible.

Pigment cells and cell populations were shown in Figure 7, (p.77), Figure 8, (p.79), and Figure 9 (p.81). Cell detail is clearest in Figure 9. Certain cell variations are plain enough. The presence, absence, or relative numbers of various pigment cells can adequately account for significant amounts of variation. But not all variations are simply demonstrable. The problems of interpretation at the cell level were not recognized at first. They are extensive and offer new opportunities for further investigation.

While wild type fish have many black melanophores on the body scales, Cambodia scales have few, though it is not known for sure whether the cells are absent or are present but unable to produce color. Blond fish have reduced numbers of black cells in the intermediate zone, but they too could have some cells that are colorless. Cell counts were not made of melanophore populations of melanos, but on the scales numbers seem about normal. However they appear to have more expanded processes full of large numbers of pigment granules. Further study is necessary to make accurate determinations.

Red cells are not found on scales of wild-type Bettas. The extended red mutant, however, has numerous red cells in the same area of the scale that has yellow and black cells. Though the color is clearly different, the cell morphology does not clearly indicate an additional cell type. In addition, the red cells have not been shown to be identical to the fin erythrophores, though superficially the color is the same. The appearance of the red cells on the body suggests that they might be

melanophores with abnormal pigment.

Non-red fish cannot be distinguished from wild type by examining pigment cells on the scale. In the fins, where one would expect to see erythrophores, I observed only what appeared to be ovoid cells containing a mass of colorless granules and lacking visible processes. My interpretation is that these abnormal cells are non-red erythrophores.

Though xanthophores are clearly present on scales of the wild type, dark or light fish which look very yellow (genotypes not all understood) seem to have far more yellow pigment. Some show only xanthophores. Others look as if the pigment in yellow cells may become orange to red. In still others, non-red erythrophores may look yellowish and, if present, supplement the yellow color of the normal xanthophore population. Ordinary xanthophores show few granules, while yellow-red cells and the erythrophores have granules which appear structurally identical to melanosomes. It is practically impossible to be sure whether an isolated cell containing reddish granules is a genuine erythrophore or a modified melanophore or a modified xanthophore.

A tabulation of mutants with probable cell population alterations is given in Table 33. A diagrammatic representation of the relationships between normal and various mutant phenotypes is shown in Figure 33.

Structural Abnormalities

A number of structural variations have been noted, some having a genetic basis, and others not, according to breeding tests.

Table 33. Summary of effects of color mutants on cell populations

Mutants	Symbol	Deviation from wild type
Cambodia	c	Melanophores mostly absent (or lacking melanin?) in integument (but not choroid).
Blond	b	Melanophore numbers decreased in fins, dorsum, and intermediate zone of body scales.
Melano	m	Melanophore numbers increased in fins (and elsewhere?), and melanin tends to be more widely and constantly dispersed in the cells.
Extended red	R	Red cells present in scales and dorsum (erythrophore invasion from fins? or reddened melanophores or xanthophores?)
Non-red	nr	Erythrophores colorless or yellowish (or absent?); no visible processes.
Variegated fins	Vf	Erythrophores absent or colorless in irregular areas of fins.
Spread iridocytes	Si	Guanine increased in scales and fins (but not in dorsum); maximum spread at maturity.
Blue	Bl	Yellow (lutein) reduced or absent? (from slime layer?) (altered iridocytes?)
Opaque	O	Guanine increased in scales and fins and dorsum, apparently develops throughout life.

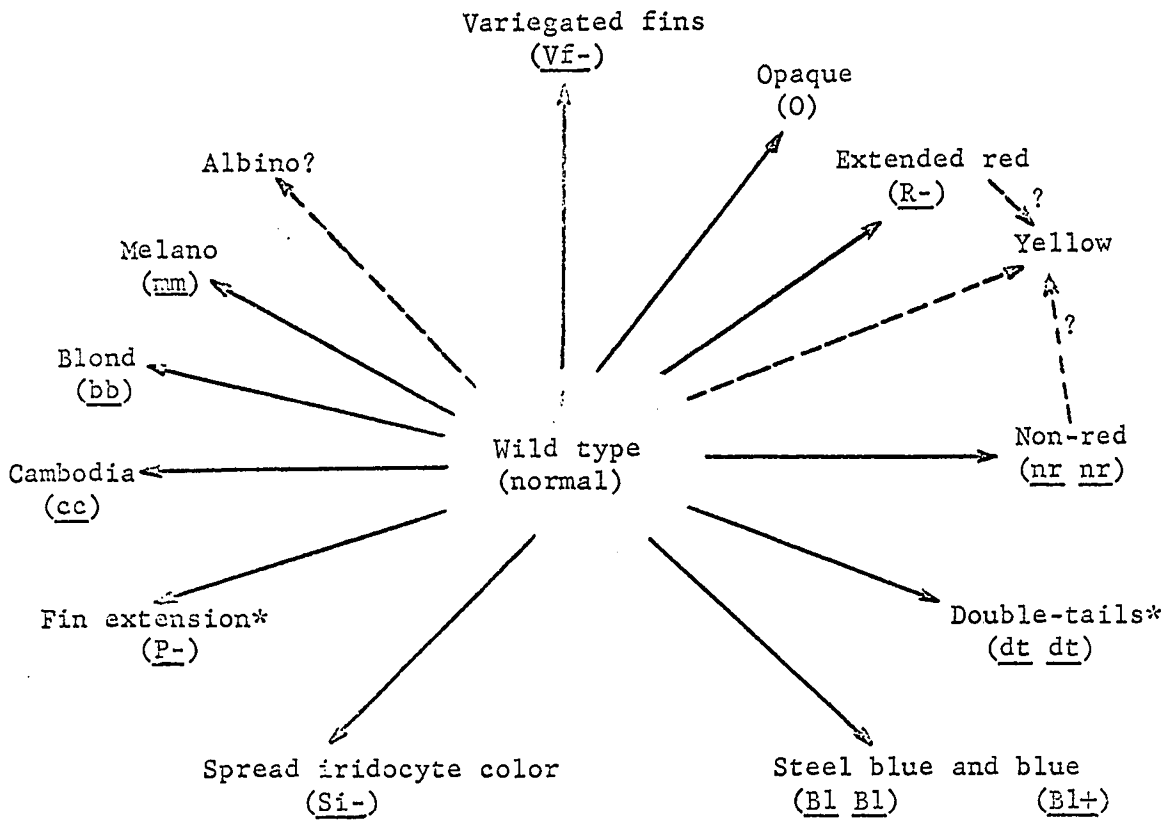


Figure 33. Relationships between Betta mutants and wild type (Includes structural mutants)
* Structural mutants to be discussed later

Genetic abnormalities

Extended fin growth (Veil-tail) The gross anatomical difference between wild type and long-finned domestic fish has already been illustrated. (Figure 2, p.13). In Bettas it is characterized by a sex difference, the males developing extension of all structural fin elements in all fins except the pectorals. Wallbrunn (1951) related that Eberhardt (1943a) demonstrated long fins (disregarding shape variation) to be controlled by a single dominant gene. I have not seen the original data. My own data from domestic X wild (short-tailed) agree with such a

conclusion (Table 34).

Table 34. Spawns from matings of long-finned X normal Bettas (various combinations)

Spawn Number	Parents: Male	Parents: Female	Progeny: Normal	Progeny: Long Fins *
107	Wild	Domestic (97) (green)	0	40-50
123	Domestic (97) (melano)	Wild	0	10
109	Wild	Wild	3	0
196 (F_2)	Normal (107-1)	Normal (107-2)	0	13
242 (F_2)	Normal (123-1)	Normal (123-2)	3	10

* Females not classified

Reciprocal crosses produced all long-finned F_1 male progeny. The F_2 from spawn 107 produced only 5 males suitable for fin classification, all with long fins. Of 13 males in the F_2 (spawn 242), of spawn 123, three were short-finned. Though data are limited all confirm the dominance of the "long" alternative.

The symbol P selected by Eberhardt probably came from the German prächtig, meaning splendid, magnificent, gorgeous, or sumptuous. The term is applicable so I suggest retention of the symbol and designation

Dorso-caudal mutant The most unusual anatomical abnormality in Bettas is a rather rare type having an unusual multiplication of dorsal fin rays and either a duplicate or bifurcate caudal fin and posterior

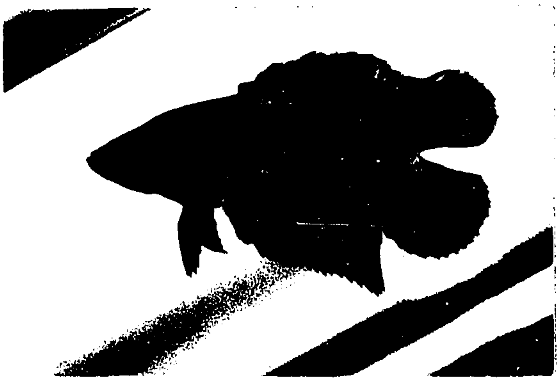
caudal peduncle (Figure 34).

Regan (1909) gives the dorsal ray numbers as 9-10 with 11 maximum in the wild type. The fish described here have 20-25 dorsal rays (Figure 34a). The caudal fin of a normal fish has 12-14 rays but each lobe of the duplicate type contains 8-10 rays. Very young fry show large wide-spread caudal rays quite unlike the normal fry.

Mr. Walt Maurus, who supplied them, says they breed true. His matings with normal fish produce all normal F_1 progeny, and matings of F_1 with abnormals produce both types of progeny. I have had only one spawn, which was between a normal F_1 male and an affected female. The young are of both types.

This anomaly has been mentioned only in hobby publications. No breeding data have been reported anywhere. I am confident of Mr. Maurus' accuracy, and our combined results demonstrate the condition to be genetic and a simple recessive.

A description by Whitern (1962) refers to these fish as "split-tails". Mr. Maurus and I both feel that "split-tail" is a misnomer. Fish with large fins frequently split their fins (separation of rays by splitting or tearing of the web membrane) and this damage may heal (Figure 34c). By contrast, the anomalous new type is a permanent developmental feature. We feel it would more properly be called "double-tail". Because of the evidence we have and the considerations mentioned I propose the symbol <u>dt</u> (for double-tail).



a

b

a. A young double-tail male showing plainly the bi-lobed caudal fin. Note the increased number of dorsal fin rays.

b. An older double-tail male. The tail is clearly double and the dorsal fin is as extensive as the anal.

c

c. A male from ordinary stocks with a mechanical split which has partially healed. Note that the dorsal fin has normal number of rays.

Figure 34. Genetic and non-genetic "Double-tail" Bettas

Non-genetic abnormalities

A number of traits were observed that produced unnatural phenotypes but which upon investigation seemed to be non-genetic. I have sub-grouped these on the basis of their being temporary (conditional or environmental) or of permanent (developmental) origin.

Conditional or environmental defects (Figure 35).

1. Split or damaged fins (Figure 35-a and b). Web membrane separations are common in some stocks. They often occur when males fight or when they injure females during a spawning drive. Poor general condition may make tissues vulerable to attack by bacterial, fungal, or other parasitic infections which then leads to tissue degeneration. Frequently the condition is minor and regeneration occurs but sometimes leads to major fin destruction and death.

2. Distention (Figure 35-c). Bettas are susceptible to "dropsy", (an example appears in Figure 31-e, p.158) where they become moribund, their scales protrude and often they die. They may also have distended abdominal areas which could result from intestinal blockage, ovarian problems or even swollen air bladder. Blocked intestines may be relieved either spontaneously or by treatment. Ovarian problems seem to result from decompositon of eggs in the ovarian tissue. Necrosis of local tissue often results, and further problems may develop. The air bladder in Bettas extends quite far back into the caudal peduncle and not uncommonly this may develop improperly or it may not fill properly. Breeders occasionally complain of "jumpers" or, "sliders", young fish which cannot

Figure 35. Abnormalities of unknown origin

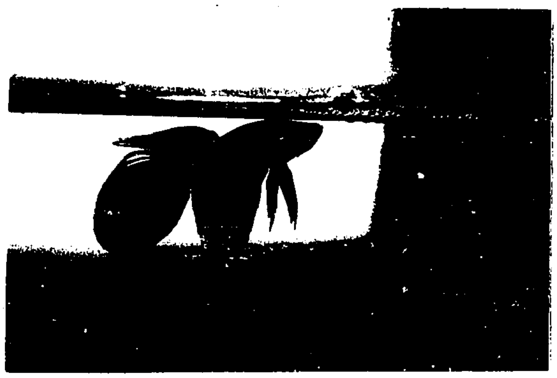
a. Male having some of the membrane absent between rays in the upper portion of the caudal fin.

b. Another specimen with a similar defect. The membrane separation is located near the center of the caudal fin. A split is present at the upper posterior rim of the caudal such as is often observed following injury.

c. A female with greatly distended abdominal area, thought to be caused by decomposition of large egg masses in the ovaries.

d. A green male with a split caudal, many misaligned rays in all median fins, jagged fin edges and deformed pelvic fins.

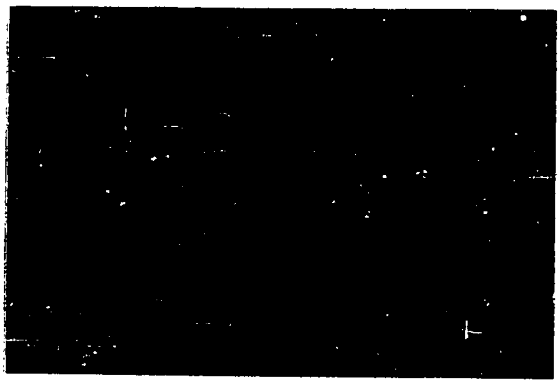
e. A "purple" male having a bent dorsal fin and rudimentary pelvic fins.


a


b


c


d


e

swim horizontally because the air bladder is not properly developed and inflated.

3. Bent, recurved, curled or misaligned fin rays (Figure 35-d and e). Frequently individuals are discovered with one or more of these defects. A single individual may have a bent fin such as the dorsal of fish in Figure 35-e. This particular condition is rarely seen and has not been reproduced. The more common poor alignments usually occur in fish (sometimes entire spawns) which grow up in poor surroundings such as fouled water.

At present there is no evidence of a genetic basis for these problems. Recurving fins (nor shown) appear to develop when growth occurs after some damage (microscopic) has been done to the free growing margins of the fins. This, I feel, follows infection of some kind, tissue damage, the formation of scar tissue, and subsequenc growth of sub-terminal tissue of the fins.

Developmental defects (Figure 36).

1. Missing or reduced caudal fin (Figure 36-a and b). Rarely, a specimen appears lacking its caudal fin. This occurred in perhaps a dozen cases during my experiments involving thousands of fish. None of these were used for breeders. However, the rarity of their appearance and the lack of relationship of their ancestry make it doubtful they have any genetic basis. The cannibalistic tendencies of spawn mates in populations suggest the possibility of loss through amputation without subsequent regeneration.

Figure 36. Abnormalities thought to be produced by faults in embryological development

a. A Cambodia male with no caudal fin. Other fins are partially folded but were normal.

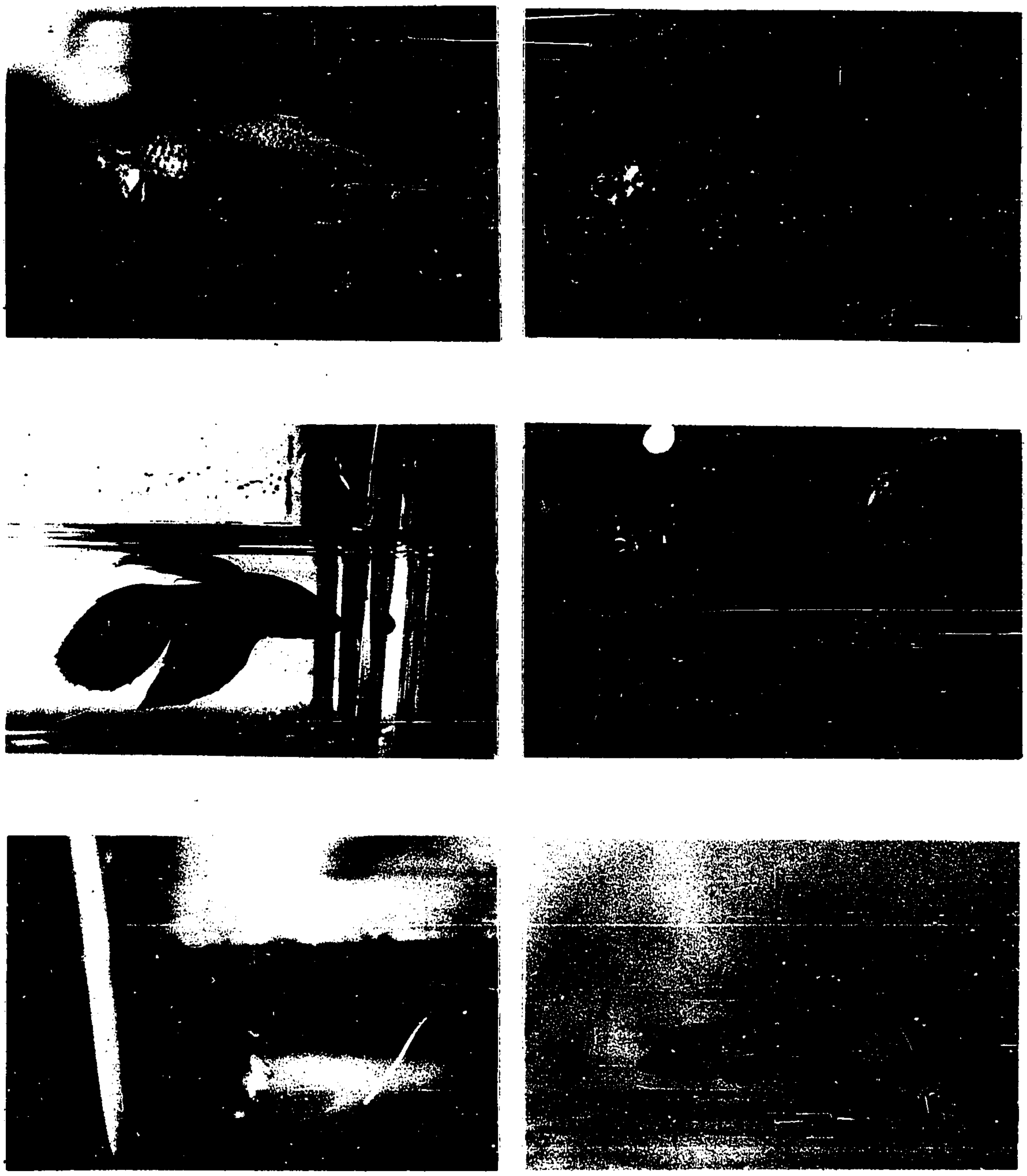
b. Another Cambodia male, this one having a less severe reduction in caudal fin size. A web membrane seems to unite caudal and anal fins.

c. An extended red male lacking the paired (ventral) fins normally located anterior to the large anal fin.

d. "Pug-nose" and other signs of retarded development thought to result from crowding.

e. A male with a notch out of the lower part of its body, involving also the anal fin.

f. A female with same abnormal notch.



a b

c d

e f

2. Missing pelvic (ventral) fins (Figure 36-c) On several occasions spawns were obtained containing numerous individuals with insufficient development of the pelvic fins. The condition was not uniform. Some fish lacked any vestige of either fin. Others had rudiments of one or both. Some had one normal fin. Wallbrunn (1951) analyzed the condition and concluded it was non-genetic. He reported an increase in dorsal ray number as an accompanying abnormality.

I agree with Wallbrunn that the condition is non-genetic. I found no consistency in the appearance of these types. They occurred in spawns from normal parents but two matings between affected individuals produced only normal progeny. Normal X abnormal matings usually produced only normal fry.

The best clue to its probable cause comes from spawn 100. The male parent did not keep eggs in a bubble nest but allowed them to lie on the bottom of the tank. A number of these were moved to a finger bowl and allowed to develop under about an inch of water. Some hatched and 19 fry were reared. Eighteen lacked pelvic fins, the other had only one.

There were several factors where teratological stimulation could occur. The eggs may require contact with air, especially at certain developmental stages, which may have been impossible as they remained on the bottom. There could be pressure differences between surface development and "bottom" development, though I consider this a minimal probability. There could have been temperature shock, since eggs were moved from a controlled 82° environment in a thermostatically controlled heated tank to an unheated finger bowl which could flucuate through several degrees with rapid changes.

3. Effects of overcrowding (Figure 36-d) In early breeding experiments I encountered what might be termed a "stunting syndrome" which resulted in phenotypes having smaller bodies, improperly developed fins, and characteristic "pug noses" apparently caused by poor development of the frontal, nasal, maxillary, and pre-maxillary complex of the superior anterior aspect of the skull.

The affected fish would recover significantly if removed to individual jars and better care, indicating an environmental basis for the problem. The skull would rarely recover however, indicating that the skull, once formed, is unable to undergo much change. "Old" or "bad" water must also be considered a factor since spawns with better care produced size-stunted fish without the other aberrations.

4. Notches in the body (Figure 36-e and f) I found two fish with a portion of the inferior part of the caudal peduncle and the corresponding portion of the large anal fin absent. A very similar condition has been observed in other aquarium fish. Both specimens were obtained from the same breeder's stock, and none were ever found from any other. A pedigree diagram is provided (Figure 37).

The two affected specimens were mated and produced spawn 124 which contained at least 50 progeny, all normal. Thus, no evidence is forthcoming of a genetic basis. Possibly mechanical damage or local tissue necrosis during early development caused the abnormality.

For reference purposes, a tabulation of all reasonably certain genetic types, including new mutants now described and suggested revisions of terminology and symbolism appears in Tables 35 and 36.

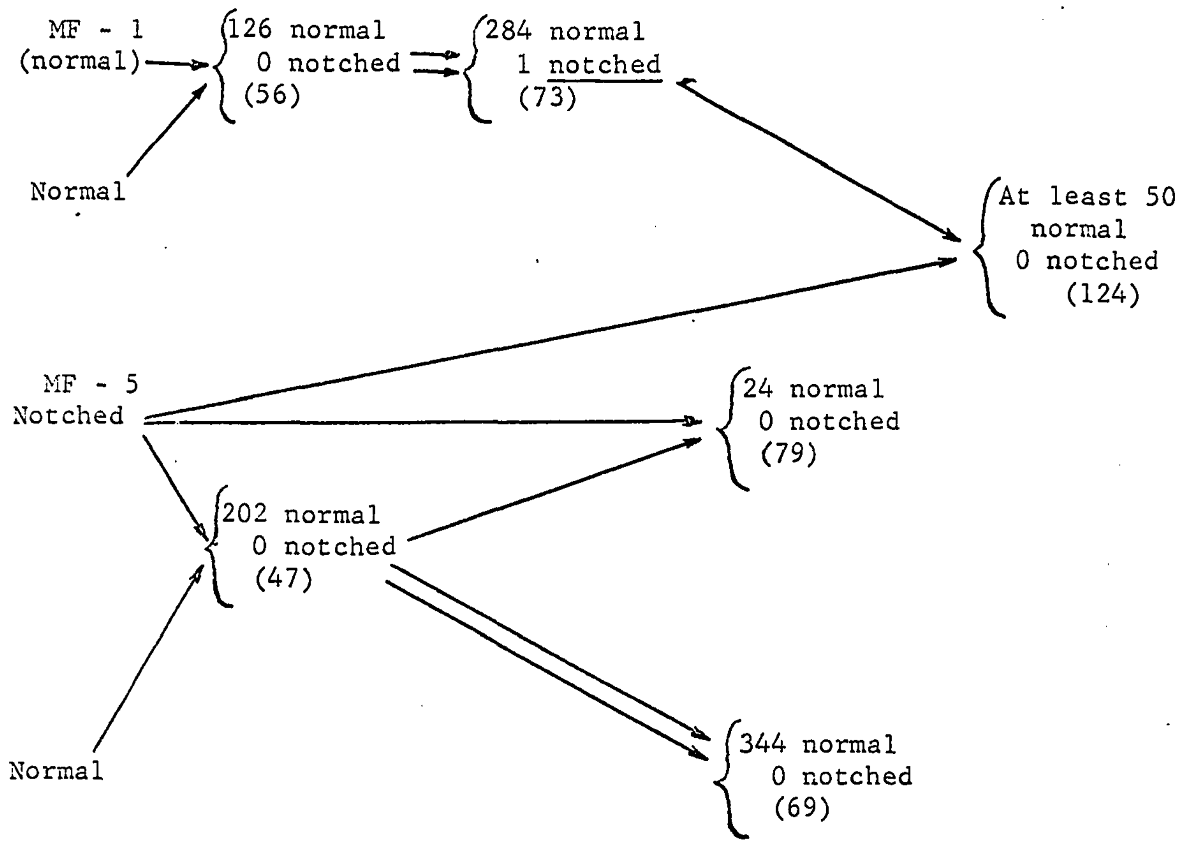


Figure 37. Pedigree chart showing origins and relationship of the two "notched" Bettas

Table 35. Summary of mutants of Betta splendens from the literature, with my revision of symbols and recommended terminology

Published mutant name	Published symbols	Described by	Date	Recommended name	Suggested mutant symbol
Previously described					
Albino	C,c	Schreit-müller	1928		
		Umrath	1939		
		Lucas	1968	Albino?	-*
Cambodia	C,c	Goodrich			
		Mercer	1934		
		Domentay	1935		
(Melano-phore re-duction)	M,m	Umrath	1939		
		Eberhardt	1941		
		Wallbrunn	1958		
		Lucas	1968	Cambodia	c
(Lipophore)	L,l	Umrath	1939		
Bright	B,b	Wallbrunn	1958		
		Lucas	1968	Blond	b
(Steel blue, blue, green)		Goodrich			
		Mercer	1934		
		Umrath	1939		
Viridens	V,v	Eberhardt	1941		
Green	G,g	Wallbrunn	1958		
		Lucas	1968	"Blue" (Steel blue, Blue)	Bl
Prächtig	P,p	Eberhardt	1941	Prächtig (splendid)	P
		Lucas	1968		
Reduced iridocytes	Ri, ri	Eberhardt	1941		
		Wallbrunn	1958		
		Lucas	1968	Spread irid-ocytes	Si

* I interpret "albinos" as Cambodia combinations or non-genetic color loss.

Table 36. Mutants of *Betta splendens* not previously described in the literature

Mutant name	Described by	Date	Recommended name	Suggested mutant symbol
Non-red	Lucas	1968	Non-red	nr
Black	Cook* Lucas	1966 1968	Melano	m
Varie-gated fins	Lucas	1968	Variegated fins	Vf
Extended red	Lucas	1968	Extended red	R
Double-tail	Maurus* Lucas	1967 1968	Double-tail	dt
Opaque	Lucas	1968	Opaque	O

* Described without mating data in hobby publications.

SEX DETERMINATION EXPERIMENT

The only previous attempt to determine environmental influences on sex ratios of Betta splendens was that of Eberhardt (1943b). His experiences suggested, as have mine, that environmental conditions may influence the sex ratios. He considered the possibility of differential sexual mortality due to aggressive differences within groups. He found a wide range of sex ratios in preliminary experiments where competition was a definite environmental factor and therefore concluded that differential mortality from this source was not important. His opinion was that exogenous masculinizing factors were involved.

Eberhardt's finding of excessive males was duplicated by Wallbrunn (1951). In my experiment 22 of 36 spawns had more than 50% males, all experimental groups had in excess of 50% males, and the total surviving progeny consisted of 827 males and 619 females, a decided masculine advantage.

Eberhardt finally concluded, with supporting data, that unfavorable environmental conditions were responsible for masculinization and optimum conditions would provide equal sex ratios but suggested that additional research was necessary to determine which feature of the environment might be responsible. My experiment was designed to 1) minimize differential mortality 2) provide uniformity of developmental environment, and 3) to investigate some more specific factors than "poor conditions" (see methods section).

The results obtained from individual matings appear in Table 37.

Table 37. Sex ratios obtained from experimental matings, expressed as males/ total survivors and percent males

Matings Combinations	Replications 1	2	3	Totals and %	Standard error
Ames water, Warm					
Old Male	21/49	18/36	34/48	73/115	± 4.5%
Young Female	42%	50%	71%	63%	
Young Male	14/29	10/50	32/50	56/129	± 4.4%
Young Female	48%	20%	64%	43%	
Young Male	42/50	37/44	38/39	117/133	± 2.8%
Old Female	84%	84%	97%	88%	
Ames water, Cool					
Old Male	19/31	20/34	42/48	81/113	± 4.2%
Young Female	61%	59%	88%	72%	
Young Male	31/44	35/50	23/45	89/139	± 4.1%
Young Female	70%	70%	51%	64%	
Young Male	19/49	27/50	16/42	62/141	± 4.3%
Old Female	39%	54%	38%	44%	
Des Moines water, Warm					
Old Male	0/27	6/29	29/47	35/103	± 4.6%
Young Female	none	21%	62%	34%	
Young Male	28/37	8/15	7/48	43/100	± 4.9%
Young Female	76%	53%	15%	43%	
Young Male	33/41	33/42	19/45	85/128	± 4.2%
Old Female	80%	79%	42%	66%	
Des Moines water, Cool					
Old Male	26/50	12/27	15/22	53/99	± 5.0%
Young Female	52%	44%	68%	54%	
Young Male	31/49	8/30	13/29	52/108	± 4.8%
Young Female	63%	27%	45%	48%	
Young Male	44/48	17/40	20/32	81/120	± 4.2%
Old Female	92%	43%	63%	68%	

The 36 spawns had male percentages ranging from 0 to 97. Among spawns having less than 6% mortality, the range in percentages of males was from 15 to 92. Since the fish were reared individually, competitive differential mortality was impossible. The highly divergent male percentages minimize the possibility of an innate difference in survival strength between the sexes.

Because of the effort to make conditions uniform within groups, one would expect replicate results to be similar, but they often differed greatly. Whether the environment was "good", or "bad", it does not seem accountable for the variation obtained.

More males were obtained from every experimental combination (Table 38). Comparisons of various groups revealed that there was no difference in the sex ratios obtained from the two temperature variables; therefore temperature was eliminated as an element for further analysis. It should be noted however that these results do not rule out the possibility of positive influence by more extreme temperature variables.

Since there were significant sex ratio differences in the other groups (Table 39) they were further analysed with a "two-way analysis of variance (with replications)" model after Freund, Livermore and Miller (1962) (Tables 40 and 41).

Table 38. Sex ratios from various combinations of variables

Variable	Males	Females	Died	% of males in survivors	Standard error
Ames Water	473	310	112	61%	± 1.7%
Des Moines Water	349	309	242	53%	± 2.0%
Cool temperature	418	302	180	58%	± 1.8%
Warm temperature	409	317	174	56%	± 1.8%
Old M X Young F	242	206	152	54%	± 2.2%
Young M X Young F	240	236	124	50%	± 2.2%
Young M X Old F	345	177	78	66%	± 2.1%

Table 39. Chi-square tests of various independent elements of the sex determination experiment

Test Elements	Chi-square	P	Level of Significance
Water variable	20.12	<0.005	Very
Temperature variable	.098	90>75	Not
Total matings combinations	26.167	<0.005	Very
O - Y matings	74.40	<0.005	Very
Y - Y matings	68.90	<0.005	Very
Y - O matings	40.32	<0.005	Very

Table 40. Table of sumes of squares used in two-way analysis of variance test with replications

	Values
Correction term $C = \frac{T^2_{\cdot\cdot}}{a \cdot b \cdot n}$	56,336
Water variable sum of squares (a) $SSA = \frac{\sum_{i=1}^{a} T_i^2}{b \cdot n} - C$	383
Mating combinations sum of squares (b) $SSB = \frac{\sum_{j=1}^{b} T_j^2}{a \cdot n} - C$	946
Mean sum of squares $SSM = \frac{\sum_{i=1}^{a} \sum_{j=1}^{b} T_{ij}^2}{n} - C$	1,654
Interaction sum of squares $SSI = SSM - SSA - SSB$	325
Replicate sum of squares $SSR = \frac{\sum_{k=1}^{n} T^2_{\cdot\cdot K}}{a \cdot b} - C$	283
Total sum of squares $SST = \sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n} X^2_{ijk} - C$	4,573
Error sum of squares $SSE = SST - SSM$	2,636

Table 41. Analysis of variance table, two-way replications

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares
Water Variable	(a-1) 2-1=12	383	383
Mating Combinations	(b-1) 3-1=2	946	473
Interaction	(a-1)(b-1) 1·2=2	325	162.5
Replicates	(n-1 3-1=2	283	141.5
Error	(n-1)(ab-1) 2·5=10	2,636	263.6
Totals	(n-1) 18-1=17	4,573	

F statistics

$F_A = \frac{MSA}{MSE} = 1.45$ df = 1.10 4.96 (value of significance)

$F_B = \frac{MSB}{MSE} = 1.79$ df = 2/10 4.10 " "

$F_R = \frac{MSR}{MSE} = .62$ df = 2/10 4.10 " "

$F_I = \frac{MSI}{MSE} = .53$ df = 2/10 4.10 " "

The F ratios obtained show no significant levels for any individual factor in the experiment. My interpretation is that, though variation exists, the test would not support a null hypothesis suggesting any one of the elements as a "causative" element. All included components must be contributing somewhat to the variation.

Further consideration of the results will require still further basic information. One additional factor which might cause variation is a seasonal "sex differential". Since seasonal data were available for the experimental matings a tabulation was made of the matings and results (Table 42).

Though matings were not established with regard to seasons, the results clearly show wide ranges of sex ratios for each season. I conclude that seasonal fluctuations, under the artificial cultural conditions, had no effect.

General observations concerning sex determination in Bettas

Some generalities may be made concerning the sex ratios of domesticated Bettas.

1. They are extremely and significantly variable.

2. There appears to be a consistent advantage to the male sex which remains unexplained.

3. Differential mortality does not appear to be a factor in the production of abnormal sex ratios.

4. Rearing of spawns in constant temperature ranges 2° F above and below the 80° F optimum did not influence the obtained sex ratios.

5. "Water conditions" appear to cause different ratios, possibly through some constituent not yet recognized.

Table 42. Matings tabulated by months of the year, information considered as % of survivors that are males

Month	Avg. %	No. of spawns	% Range	No. above 50%	No. below 50%
Jan					
Feb	56	13	00 to 92	8	5
Mar	62	2	62 to 63	2	0
Apr	54	5	27 to 80	3	2
May	64	2	59 to 70	2	0
Jun	71	1		1	0
Jul					
Aug	21	1		0	1
Sep	66	8	44 to 97	6	2
Oct					
Nov	40	3	15 to 84	1	2
Dec	42	1		0	1
Totals	56	36	00 to 97	23	13

6. Mating combinations involving parents' age variations can produce abnormal sex ratios.

7. There is some evidence that a young male, older female combination may result in a higher male percentage.

The possibility that Bettas possess a labile sex-determining system, as indicated by previous reports, still exists, and seems reinforced by my findings. It is possible that exogenous factors are critical. Further

investigations are required of the possibilities of exogenous factors not yet considered. Specific constituents of water must be suspected because of their proven effect. (Cases of _Bonellia_ and _Tigriopus_, p.50). The crowding effect in oysters suggests another possibility that closely parallels the Betta case. Hunger caused by crowding, if truly a factor, certainly could not be discounted. Many large spawns have numerous "stunted" individuals which no doubt suffer from, among other things, food deficiencies.

The lack of conclusive evidence for a cytological genetic sex-determining system remains a handicap. It is possible that Bettas may have a "borderline" genetic sex determining system and that such fish as the Betta, _Xiphophorus helleri_ and others represent a slight step up, in this respect, from more clearly protogynous animals. This idea is supported by the normal development through undifferentiated stages to final definitive forms and the occurrence of female-to-male sex reversal but no instances of the reverse.

SUMMARY

The objective of this study was to survey the literature on the Genetics of the Siamese Fighting Fish, Betta splendens Regan, as well as to make breeding-test analyses of available variant types.

In order to clarify the genetics of the numerous colorations, resolution of the mutant genes was undertaken in relation to the pigmentation components which they affect. At first wild-type specimens were not obtainable in the U.S., and a synthetic wild-type was used as a standard of reference. Later actual wild fish were imported from Germany and South Vietnam.

Color mutants identified were as follows: 1) Cambodia, symbol c, causing nearly complete failure of melanophore development except in the choroid; 2) blond, b, causing reduction in number of melanophores especially on the dorsum; 3) spread iridocytes, Si, causing more widespread occurrence of guanine crystals on the scales and fins; 4) blue, Bl, incompletely dominant, the homozygous mutant being "steel blue"; this is a change in reflective effect of the iridocytes, but may possibly be a property of the slime layer; 5) melano, m, causing abnormal expansion of the melanophore processes; the eggs of mm females fail to produce embryos; 6) non-red, nr, causing erythrophores to be degenerate except in rare flecks or streaks; 7) extended red, R, causing erythrophores (or red melanophores?) to be present in body scales and over the head, best seen in the adult males; 8) variegated fins, Vf, causing failure of red pigment-cell development in portions of the fins; and 9) opaque, O, causing extra deposition of creamy matter (guanine?)

throughout the integument, and often swollen eyes.

"Yellow" Bettas seem to be combinations of c with nr and (or R). No true genetic albino type has been identified. Other combination effects, interactions, possible linkages, etc., were considered but much more work on these remains to be done.

A new structural mutant, "double-tail", is reported, and the symbol dt proposed. This causes dorso-ventral doubling of the tail, as well as the extra ray development in the dorsal fin. The only other known structural mutant is the prevalent long-fin type, P, expressed chiefly in adult males. Several non-genetic structural abnormalities were studied briefly. Also an apparently non-genetic color-loss effect was observed in a number of specimens; pigment cells, especially melanophores, would degenerate over part or all of the body and even in the choroid, giving more or less albinotic effects. Regeneration sometimes occurred.

During the early breeding tests highly aberrant sex ratios called attention to the question of sex-determination. An experiment was set up to test the possible influence of kind of water, of temperature, and of parental age on sex-ratio. Extraordinary fluctuations were common even with closely controlled conditions, and no certain evidence was obtained as to cause. In general there was a tendency to produce excess males. The Betta apparently does not have simple genetic sex determination.

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